

ANTI-INTEGRIN BETA7 ANTIBODY FORMULATIONS AND DEVICES**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims priority to U.S. Provisional Patent Application Serial No. 63/059,427, filed July 31, 2020, the content of which is incorporated by reference in its entirety, and to which priority is claimed.

FIELD

Formulations comprising an anti-integrin beta7 antibody or an antigen-binding fragment thereof are provided, including pharmaceutical formulations and devices comprising such formulations and methods of using such formulations and devices.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 27, 2021, is named 00B2061132SL.txt and is 13,697 bytes in size.

BACKGROUND

The integrins are $\alpha\beta$ heterodimeric cell surface receptors involved in numerous cellular processes from cell adhesion to gene regulation (Hynes *Cell* (1992);69:11-25; and Hemler, *Annu. Rev. Immunol.* (1990), 8:365-368). Several integrins have been implicated in disease processes and have generated widespread interest as potential targets for drug discovery (Sharar *et al.*, *Springer Semin. Immunopathol.* (1995);16:359-378). In the immune system, integrins are involved in leukocyte trafficking, adhesion and infiltration during inflammatory processes (Nakajima *et al.*, *J. Exp. Med.* (1994);179:1145-1154). Differential expression of integrins regulates the adhesive properties of cells and different integrins are involved in different inflammatory responses (Butcher *et al.*, *Science* (1996);272:60-66. The beta7 integrins (i.e., $\alpha4\beta7$ and $\alpha\text{phaE}\beta7$) are expressed primarily on monocytes, lymphocytes, eosinophils, basophils, and macrophages but not on neutrophils (Elices *et al.*, *Cell* (1990);60:577-584). The primary ligands for $\alpha4\beta7$ integrin are the endothelial surface proteins mucosal addressin cell adhesion molecule (MAdCAM) and vascular cell adhesion molecule (VCAM-1) (Makarewicz *et al.*, *J. Biol. Chem.* (1994);269:4005-4011). The binding of the $\alpha4\beta7$ to MAdCAM and/or VCAM expressed on high endothelial venules (HEVs) at sites of inflammation results in firm adhesion of the leukocyte to the endothelium followed by extravasation into the inflamed tissue (Chuluyan *et al.*, *Springer Semin. Immunopathol.*,

1995, 16:391404). Monoclonal antibodies directed against $\alpha 4\beta 7$, MAdCAM or VCAM have been shown to be effective modulators in animal models of chronic inflammatory diseases such as asthma (Laberge *et al.*, *Am. J. Respir. Crit. Care Med.* (1995);151:822-829.), rheumatoid arthritis (Barbadillo *et al.*, *Springer Semin. Immunopathol.* (1995);16:375-379), colitis (Viney, *J. Immunol.* (1996);157: 2488-2497) and inflammatory bowel diseases (Podalski, *N. Eng. J. Med.* (1991);325:928-937; Powrie *et al.*, *Ther. Immunol.* (1995);2:115-123).

Humanized anti-integrin beta7 antibodies and antigen-binding fragments thereof have been described. *See, e.g.*, Intn'l Patent Publication No. WO2006/026759. One particular anti-integrin beta7 antibody, etrolizumab, has been clinically investigated for treating gastrointestinal inflammatory disorders, e.g., inflammatory bowel disease, e.g., ulcerative colitis and Crohn's disease. The results of a Phase 1 study of etrolizumab in moderate to severe ulcerative colitis were described in Rutgeerts PJ, et al. *Gut* 2013;62:1122–1130 reporting no clinically significant safety signals observed. The results of a global multicenter Phase 2 study of etrolizumab in moderate to severe ulcerative colitis showed evidence of clinical efficacy of etrolizumab treatment as measured by induction of clinical remission (Vermeire, S. et al., *Lancet* 2014; 384: 309–18). In addition, clinically meaningful remission was observed following treatment of moderate to severe Crohn's Disease patients with etrolizumab (Sandborn et al., presentation entitled "Etrolizumab as Induction Therapy in Moderate to Severe Crohn's Disease: Results from Bergamot Cohort 1," presented at United European Gastroenterology Week Congress, Oct. 28-Nov. 2, 2017). Accordingly, etrolizumab has demonstrated promise as a therapeutic treatment option in inflammatory bowel diseases and further studies are ongoing to refine the safety and efficacy profile of etrolizumab.

In each of the reported studies to date, etrolizumab was administered by a health care provider in a clinical setting either intravenously or subcutaneously. For subcutaneous administration, a vial and syringe with a vial concentration of 150 mg/ml was used. Because inflammatory bowel diseases such as ulcerative colitis and Crohn's Disease are chronic diseases, long-term therapeutic treatment with etrolizumab may be needed. For optimal patient convenience and compliance among other advantages, self-administration of etrolizumab or administration in the home by a caregiver or healthcare professional is desirable. Accordingly, development of self-administration devices and formulations of etrolizumab compatible with such devices would be advantageous.

Because proteins, including antibodies such as etrolizumab, are larger and more complex than traditional organic and inorganic drugs (e.g., possessing multiple functional groups in addition to complex three-dimensional structures), the formulation of such proteins poses special problems. For a protein to remain biologically active, a formulation must preserve intact the conformational integrity of at least a core sequence of the protein's amino acids while at the same time protecting the protein's multiple functional groups from degradation. Degradation pathways for proteins can involve chemical instability (e.g., any process which involves modification of the protein by bond formation or cleavage resulting in a new chemical entity) or physical instability (e.g., changes in the higher order structure of the protein). Chemical instability can result from deamidation, racemization, hydrolysis, oxidation, beta elimination or disulfide exchange. Physical instability can result from denaturation, aggregation, precipitation or adsorption, for example. The three most common protein degradation pathways are protein aggregation, deamidation and oxidation. Cleland et al Critical Reviews in Therapeutic Drug Carrier Systems 10(4): 307-377 (1993).

High concentration (e.g., > 100 mg/mL) liquid antibody formulations are desirable, for example, for routes of therapeutic administration or for therapeutic applications where small volumes of drug product are advisable, for example, for subcutaneous injection including, for example, using a prefilled syringe or self-administration device. High concentration antibody formulations, however, pose numerous challenges and problems including challenges and problems associated with use of prefilled syringes or self-administration devices. One problem is instability due to the formation of particulates. With reconstituted liquid formulations, this problem has been addressed through the use of surfactants (e.g., a polysorbate), but surfactants are sometimes thought unsuitable for liquid formulations, because they render further processing difficult. Moreover, surfactants further do not reduce the increased viscosity caused as a result of numerous intermolecular interactions from the macromolecular nature of antibodies.

Selection of pH and optimal excipients is important for preventing particle formation resulting from polysorbate-induced degradation, preventing isomerization of certain amino acids and formation of undesirable intermediates and for extending shelf-life in addition to providing advantages for manufacturing. Selection of pH and optimal excipients is also important for development of formulations, e.g., for compatibility with storage conditions, for administration by prefilled syringe, including prefilled syringe

containing devices such as a prefilled syringe with a needle safety device or an autoinjector or self-administration devices, for example, to ensure compatibility with device components and to provide low injection forces.

It would be highly advantageous to have formulations comprising an anti-beta7 antibody, including etrolizumab, having extended stability and low viscosity at high antibody concentrations. High antibody concentration formulations having such properties would be highly advantageous for certain routes of administration, e.g., for subcutaneous administration, including use with prefilled syringes and self-administration devices. The formulations provided herein address these needs and provide other useful benefits.

It would be highly advantageous to have formulations comprising an anti-beta7 antibody having extended stability and low viscosity at high antibody concentrations. High antibody concentration formulations having such properties would be highly advantageous for certain routes of administration, e.g., for subcutaneous administration. The formulations provided herein address these needs and provide other useful benefits.

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

SUMMARY

The formulations of the present disclosure are based, at least in part, on the discovery that an anti-integrin beta7 antibody described herein, etrolizumab, can be formulated at a high concentration (about > 100 mg/mL) in a histidine buffer, and arginine succinate, and a surfactant and that such high antibody concentration formulation is of low viscosity, has extended physical and chemical stability and maintains potency. The presently disclosed formulations are optimally compatible for self-administration devices such as a prefilled syringe with a needle safety device (PFS-NSD). In certain embodiments, the prefilled syringe is assembled into an autoinjector. The presently disclosed formulation can be packaged into subcutaneous administration devices as described herein with maintenance of, for example, product stability and other desirable attributes. Formulations of the present disclosure are useful for, e.g., the treatment of a gastrointestinal inflammatory disorder, e.g., an inflammatory bowel disease, e.g., ulcerative colitis and Crohn's disease.

Accordingly, in one aspect, a formulation comprising an anti-integrin beta7 antibody or an antigen-binding fragment thereof is provided. In certain embodiments, the concentration of the antibody or antigen-binding fragment thereof in the formulation

is at least about 100 mg/mL and the viscosity of the formulation is less than about 20 centipoise (cP) at 25°C. In certain embodiments, the viscosity of the formulation is less than about 7 cP at 25°C.

In certain embodiments, the anti-integrin beta7 antibody is a monoclonal antibody. In certain embodiments, the anti-integrin beta7 antibody is a humanized antibody. In certain embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

- 10 (i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;
- (ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO:2;
- (iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3;
- (iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4;
- (v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and
- 15 (vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or SEQ ID NO:7. In certain embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 8, and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9. In certain embodiments, the anti-
- 20 integrin beta7 antibody or antigen-binding fragment thereof comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 11. In certain embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10
- 25 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 12. In certain embodiments, the anti-integrin beta7 antibody is etrolizumab. In certain embodiments, the concentration of the anti-integrin beta7 antibody or antigen-binding fragment thereof in the formulation is between about 100 mg/ml and about 220 mg/ml. In certain embodiments, the concentration of the anti-integrin beta7 antibody or antigen-
- 30 binding fragment thereof in the formulation is about 150 mg/ml.

In certain embodiments, the pH of the formulation is greater than 5.0 and up to 7.0. In certain embodiments, the pH of the formulation is greater than 5.5. In certain embodiments, the pH of the formulation is between 5.6 and 6.1. In certain embodiments, the pH of the formulation is 5.8, between 5.7 and 5.9, or between 5.75 and 5.85.

In certain embodiments, the formulation comprises arginine-succinate. In certain
embodiments, the concentration of the arginine succinate in the formulation is between
about 100 mM and about 300 mM. In certain embodiments, the concentration of the
arginine succinate in the formulation is between about 150 mM and about 300 mM. In
5 certain embodiments, the concentration of the arginine succinate in the formulation is
between about 150 mM and about 250 mM. In certain embodiments, the concentration
of the arginine succinate in the formulation is about 200 mM.

In certain embodiments, the formulation further comprises a surfactant, and the
concentration of the surfactant in the formulation is greater than 0.01% weight/volume
10 (w/v) and up to about 1% w/v. In certain embodiments, the concentration of the
surfactant in the formulation is between 0.03% w/v and 0.06% w/v. In certain
embodiments, the concentration of the surfactant in the formulation is 0.04% w/v or
about 0.04% w/v. In certain embodiments, the surfactant is polysorbate 20.

In certain embodiments, the formulation further comprises histidine. In certain
15 embodiments, the concentration of the histidine in the formulation is between about 5
mM and about 40 mM. In certain embodiments, the concentration of the histidine in the
formulation is 20 mM or about 20 mM.

In certain embodiments, the formulation has extended stability. In certain
embodiments, the anti-integrin beta7 antibody is stable for at least about seven years at -
20 °C. In certain embodiments, the anti-integrin beta7 antibody or antigen-binding
20 fragment thereof is stable for at least about one year at 5 °C. In certain embodiments, the
anti-integrin beta7 antibody or antigen-binding fragment thereof is stable for at least
about five years at 5 °C. In certain embodiments, the anti-integrin beta7 antibody or
antigen-binding fragment thereof is stable for about six years at 5 °C.

25 In certain embodiments, the anti-integrin beta7 antibody or antigen-binding
fragment thereof is stable for at least about 1 day at room temperature. In certain
embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof is
stable for up to about one month at room temperature.

In another aspect, the present disclosure provides a formulation comprising an
30 anti-integrin beta7 antibody or an antigen-binding fragment thereof in 20 mM or about
20 mM histidine buffer, pH 5.8, 0.04% polysorbate 20, and 200 mM or about 200 mM
arginine succinate, wherein the concentration of the anti-integrin beta7 antibody is about
150 mg/ml, and wherein the anti-integrin beta7 antibody comprises three light chain
hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain

HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

- (i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;
- (ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO:2;
- (iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3;
- 5 (iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4;
- (v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and
- (vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or SEQ ID NO:7.

In certain embodiments, the formulation has a pH of between 5.7 and 5.9 or
10 between 5.75 and 5.85. In certain embodiments, the formulation comprises 0.04% polysorbate 20 or about 0.04% polysorbate 20.

The presently disclosed subject matter provides a formulation comprising an anti-integrin beta7 antibody, in 20 mM histidine buffer or about 20 mM histidine buffer, pH 5.8 or pH between 5.7 and 5.9 or pH between 5.75 and 5.85, 0.04% polysorbate 20 or
15 about 0.04% polysorbate 20, and 200 mM arginine succinate or about 200 mM arginine succinate, and wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein: (i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1; (ii) the HVR-L2 comprises the amino acid
20 sequence set forth in SEQ ID NO:2; (iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3; (iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4; (v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and (vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or SEQ ID NO:7. In certain embodiments, the anti-integrin beta7 antibody
25 comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 11. In certain embodiments, the anti-integrin beta7 antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 12. In certain embodiments, the anti-
30 integrin beta7 antibody is etrolizumab.

In certain embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 8, and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9. In certain embodiments, the anti-

integrin beta7 antibody or antigen-binding fragment thereof comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 11. In certain embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof
5 comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 12.

In still a further aspect, an article of manufacture comprising a subcutaneous administration device is provided. In certain embodiments, the subcutaneous administration device delivers to a subject a flat dose of an anti-integrin beta7 antibody
10 or an antigen-binding fragment thereof. In certain embodiments, the flat dose is about 100 mg. In certain embodiments, the flat dose is 105 mg. In certain embodiments, the flat dose is about 200 mg. In certain embodiments, the flat dose is 210 mg. In certain embodiments, the anti-integrin beta7 antibody is etrolizumab. The anti-integrin beta7 antibody or antigen-binding fragment thereof in the subcutaneous administration device
15 is formulated as described above such that it is provided in a stable pharmaceutical formulation.

In certain embodiments, the subcutaneous administration device is a needle safety device. In certain embodiments, the needle safety device comprises a prefilled syringe. In certain embodiments, the anti-integrin beta7 antibody is stable at the subcutaneous
20 administration device for at least about 60 months at 5°C, or at least about 3 months at 25°C. In certain embodiments, the prefilled syringe comprises a glass barrel, a plunger stopper, a needle, and needle shield or a tip cap. In certain embodiments, the needle shield is a rigid needle shield. In certain embodiments, the rigid needle shield comprises a rubber formulation having low zinc content. In certain embodiments, the rigid needle
25 shield comprises an elastomeric component, and a rigid shield. In certain embodiments, the prefilled syringe is assembled into an autoinjector.

In certain embodiments, the volume of the formulation contained in the prefilled syringe is between about 0.5 mL and about 2.0 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is between about 0.5 mL and about
30 1.0 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is about 0.7 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is about 0.75 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is about 1.0 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is

between about 1.0 mL and about 1.5 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is about 1.4 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is about 1.5 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is about 1.45 mL. In certain embodiments, the prefilled syringe has a syringe capacity of 1 mL. In certain embodiments, the prefilled syringe has a syringe capacity of 2.25 mL.

In certain embodiments, the prefilled syringe comprises silicone oil. In certain embodiments, the amount of silicone oil in the prefilled syringe is not greater than about 1 mg. In certain embodiments, the amount of silicone oil in the prefilled syringe is between about 0.1 mg and about 1 mg. In certain embodiments, wherein the amount of silicone oil in the prefilled syringe is between about 0.2 mg and about 0.6 mg. In certain embodiments, the amount of silicone oil in the prefilled syringe is between about 0.5 mg and 0.9 mg.

In certain embodiments, the needle safety device has an injection forces that is not greater than about 50 Newton (N). In certain embodiments, the needle safety device has an injection force that is not greater than about 35 Newton (N). In certain embodiments, the needle safety device has an injection force that is not greater than about 33 Newton (N).

The presently disclosed subject matter provides an article of manufacture comprising about 0.7 mL of a formulation and a subcutaneous administration device, wherein

(a) the formulation comprises an anti-integrin beta7 antibody, in 20 mM histidine buffer or about 20 mM histidine buffer, pH 5.8 or pH between 5.7 and 5.9 or pH between 5.75 and 5.85, 0.04% polysorbate 20 or about 0.04% polysorbate 20, and 200 mM arginine succinate or about 200 mM arginine succinate, and wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

- (i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;
- (ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO:2;
- (iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3;
- (iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4;
- (v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and
- (vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or

SEQ ID NO:7, and

(b) the subcutaneous administration device is a needle safety device that comprises 1 mL of a prefilled syringe with a syringe capacity of 1 mL.

In certain embodiments, the anti-integrin beta7 antibody is present in the formation at a concentration of 150 mg/mL or about 150 mg/ml.

The presently disclosed subject matter provides an article of manufacture comprising about 1.4 mL of a formulation and a subcutaneous administration device, wherein

(a) the formulation comprises an anti-integrin beta7 antibody, in 20 mM histidine buffer or about 20 mM histidine buffer, pH 5.8 or pH between 5.7 and 5.9 or pH between 5.75 and 5.85, 0.04% polysorbate 20 or about 0.04% polysorbate 20, and 200 mM arginine succinate or about 200 mM arginine succinate, and wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

(i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;

(ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO:2;

(iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3;

(iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4;

(v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and
(vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or SEQ ID NO:7, and

(b) the subcutaneous administration device is a needle safety device that comprises 1 mL of a prefilled syringe with a syringe capacity of 2.25 mL.

In certain embodiments, the anti-integrin beta7 antibody is present in the formation at a concentration of 150 mg/mL or about 150 mg/ml.

The presently disclosed subject matter further provides autoinjectors comprising the article of manufacture disclosed herein.

The presently disclosed subject matter provides an autoinjector comprising an article of manufacture comprising about 0.7 mL of a formulation and a subcutaneous

administration device, wherein

(a) the formulation comprises an anti-integrin beta7 antibody, in 20 mM histidine buffer or about 20 mM histidine buffer, pH 5.8 or pH between 5.7 and 5.9 or pH between 5.75 and 5.85, 0.04% polysorbate 20 or about 0.04% polysorbate 20, and 200 mM arginine succinate or about 200 mM arginine succinate, and wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

- (i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;
- (ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO:2;
- (iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3;
- (iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4;
- (v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and
- (vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or SEQ ID NO:7, and

(b) the subcutaneous administration device is a needle safety device that comprises 1 mL of a prefilled syringe with a syringe capacity of 1 mL.

In certain embodiments, the anti-integrin beta7 antibody is present in the formulation at a concentration of 150 mg/mL or about 150 mg/mL.

The presently disclosed subject matter provides an autoinjector comprising article of manufacture comprising about 1.4 mL of a formulation and a subcutaneous administration device, wherein

(a) the formulation comprises an anti-integrin beta7 antibody, in 20 mM histidine buffer or about 20 mM histidine buffer, pH 5.8 or pH between 5.7 and 5.9 or pH between 5.75 and 5.85, 0.04% polysorbate 20 or about 0.04% polysorbate 20, and 200 mM arginine succinate or about 200 mM arginine succinate, and wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

- (i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID

NO:1;

(ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID

NO:2;

(iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID

5 NO:3;

(iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID

NO:4;

(v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and

(vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID

10 NO:6 or SEQ ID NO:7, and

(b) the subcutaneous administration device is a needle safety device that comprises 1 mL of a prefilled syringe with a syringe capacity of 2.25 mL.

In certain embodiments, the anti-integrin beta7 antibody is present in the formulation at a concentration of 150 mg/mL or about 150 mg/ml.

15 In yet another aspect, a method of treating a gastrointestinal inflammatory disorder in a subject is provided. In certain embodiments, the method comprises administering to the subject an effective amount of any of the above formulations.

In still yet another aspect, methods of administering subcutaneously a formulation comprising an anti-integrin beta7 antibody or an antigen-binding fragment thereof are provided. Such methods comprise administering subcutaneously any of the formulations described above. In certain embodiments, the methods comprise a subcutaneous administration device according to any of the devices described above. In certain embodiments, the method comprises the autoinjector disclosed herein. In certain embodiments, the administering results in mild pain or no pain. In certain embodiments, 20 the administering results in a transient and mild injection site reaction. In certain embodiments, the full dose is administered or at least 90% of the full dose is administered. In certain embodiments, the administering provides an equivalent exposure to etrolizumab compared to a prefilled syringe with needle safety device.

25 The presently disclosed subject matter also provides uses of the formulation, the article of manufacture, or the autoinjector disclosed herein in a therapy.

30 Furthermore, the presently disclosed subject matter provides uses of the formulation, the article of manufacture, or the autoinjector disclosed herein in treating a gastrointestinal inflammatory disorder in a subject. In certain embodiments, the

gastrointestinal inflammatory disorder is an inflammatory bowel disease. In certain embodiments, the inflammatory bowel disease is ulcerative colitis or Crohn's disease.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts solubility curves for lauric acid, myristic acid, and palmitic acid as a function of pH and polysorbate 20 (PS20) concentration as described in Example 1.

Figure 2 depicts impact of arginine on solution viscosity as described in Example 1.

Figure 3 depicts impact of protein concentration on viscosity of formulations comprising 200mM arginine succinate as described in Example 1.

Figure 4 depicts the change in the percentage of high molecular weight species (HMWS) of etrolizumab in pre-filled syringes with varying quantities of silicone oil as described in Example 1. The term “high molecular weight species (HMWS)” and the term “high molecular weight forms (HMWF)” are used interchangeability herein.

Figure 5 depicts the percentage of HMWS of etrolizumab in varying concentrations of tungsten over time as described in Example 1.

Figure 6 depicts the impact of zinc on the viscosity of etrolizumab at varying protein concentrations in formulations comprising 20 mM histidine, 200 mM arginine succinate, pH 5.8 at 25°C as described in Example 1.

Figure 7 shows protein aggregate formation by 50 mM zinc and 150 mg/mL etrolizumab at room temperature as described in Example 1.

Figure 8 depicts the HMWS formation by 10 mM zinc and 10 mg/mL or 50 mg/mL etrolizumab at 40°C as described in Example 1.

Figure 9 depicts the impact of histidine on HMWS formation in formulations comprising 10 mM zinc and 10 mg/mL etrolizumab at 40°C as described in Example 1.

Figure 10 depicts the impact of succinate on HMWS formation in formulations comprising 10 mM zinc and 10 mg/mL etrolizumab at 40°C as described in Example 1.

Figure 11 shows varying concentrations of histidine and succinate and the combined impact on HMWS formation in formulations comprising 10 mM zinc and 50 mg/mL etrolizumab at 40°C as described in Example 1.

Figure 12 shows exemplary prefilled syringes (top two) and autoinjector (last) as described in Example 2.

Figure 13 shows prefilled autoinjector of etrolizumab as described in Example 2.

Figure 14 shows autoinjector (AI) tolerability and human factors study design as described in Example 2.

Figure 15 shows graph indicating pain over time by intensity (7-point Visual Descriptive Scale) as described in Example 2.

5 Figure 16 depicts graphs indicating pain over time by injection site (7-point Visual Descriptive Scale) as described in Example 2.

Figure 17 shows the two-part pharmacokinetic bridging study design as described in Example 2. *Justification of geometric mean ratio (GMR) of 1.15 as decision point was based on the assumption that at 15% difference between prefilled syringe with
10 needle safety device (PFS-NSD) was likely to be real and, therefore, the study would be unable to demonstrate bioequivalence. †Adjustment to N driven by GMR from pilot study. AI autoinjector; AUC area under the curve; C_{max} maximum serum concentration of etrolizumab.

Figure 18 shows participant disposition as described in Example 3. AI
15 autoinjector, AUC area under the curve, PFS-NSD prefilled syringe with needle safety device, PK pharmacokinetic, SC subcutaneous. ^aExcluded because of eligibility criteria (weight restriction). Participants were excluded from specific PK analyses because of insufficient PK data for calculations.

Figure 19 shows the impact of body weight on etrolizumab C_{max} (top) or AUC_{0-inf}
20 (Bottom) as described in Example 3. AI autoinjector, AUC area under the curve, AUC_{0-inf} AUC extrapolated to infinity, C_{max} maximum concentration, PFS-NSD prefilled syringe with needle safety device, SC subcutaneous.

Figure 20 shows etrolizumab serum concentrations over time with AI and PFS-NSD on a linear scale (A) and semi-logarithmic scale (B) in the pivotal study as
25 described in Example 3. AI autoinjector, PFS-NSD prefilled syringe with needle safety device.

Figure 21 shows etrolizumab serum concentrations over time by ADA status with the AI (N=73 total, with 20 subjects being ADA positive, top) and PFS-NSD (N=75, with 24 subjects being ADA positive, bottom) as described in Example 3. ADA antidrug
30 antibody, AI autoinjector, PFS-NSD prefilled syringe with needle safety device.

Figure 22 shows the different force definitions during injection as described in Example 4.

DETAILED DESCRIPTION

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton et al., Dictionary of Microbiology and Molecular Biology
5 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), and March, Advanced Organic Chemistry Reactions, Mechanisms and Structure 4th ed., John Wiley & Sons (New York, N.Y. 1992), provide one skilled in the art with a general guide to many of the terms used in the present application.

CERTAIN DEFINITIONS

10 For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with any document incorporated herein by reference, the definition set forth below shall control.

As used in this specification and the appended claims, the singular forms “a,”
15 “an” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a protein” or an “antibody” includes a plurality of proteins or antibodies, respectively; reference to “a cell” includes mixtures of cells, and the like.

As used herein, the term “about” or “approximately” means within an acceptable
20 error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” can mean within 3 or more than 3 standard deviations, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more
25 preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to “about
30 X” includes description of “X.”

The term “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile.

“Pharmaceutically acceptable” excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

5 A “sterile” formulation is aseptic or free or essentially free from all living microorganisms and their spores.

A “frozen” formulation is one at a temperature below 0°C. Generally, the frozen formulation is not freeze-dried, nor is it subjected to prior, or subsequent, lyophilization. In certain embodiments, the frozen formulation comprises frozen drug substance for storage (in stainless steel tank) or frozen drug product (in final vial configuration).

10 A “stable” formulation is one in which the protein therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. In certain embodiments, the formulation essentially retains its physical and chemical stability, as well as its biological activity upon storage. The storage period is generally selected based on the intended shelf-life of the formulation.

15 As used herein, a formulation having “extended stability” means one in which the protein therein essentially retains its physical stability, chemical stability, and biological activity upon storage at 5°C for one year or more. In certain embodiments, the storage is at 5 °C for one year or more. In certain embodiments, the storage is at 5 °C for up to five years or six years. In certain embodiments, the anti-integrin beta7 antibody is stable for
20 at least about 1 day at room temperature. In certain embodiments, the anti-integrin beta7 antibody is stable for up to about one month at room temperature. As used herein, the room temperature is between about 20 and about 22 °C. In certain embodiments, the room temperature is about 20 °C.

A protein “retains its physical stability” in a pharmaceutical formulation if it
25 shows no signs or very little of aggregation, precipitation and/or denaturation upon visual examination of color and/or clarity, or as measured by UV light scattering or by size exclusion chromatography.

A protein “retains its chemical stability” in a pharmaceutical formulation, if the chemical stability at a given time is such that the protein is considered to still retain its
30 biological activity as defined below. Chemical stability can be assessed by detecting and quantifying chemically altered forms of the protein. Chemical alteration may involve size modification (e.g. clipping) which can be evaluated using size exclusion chromatography, SDS-PAGE and/or matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOF MS), for example. Other types of chemical

alteration include charge alteration (e.g. occurring as a result of deamidation) which can be evaluated by ion-exchange chromatography or imaged capillary isoelectric focusing (icIEF), for example.

5 An antibody “retains its biological activity” in a pharmaceutical formulation, if the biological activity of the antibody at a given time is within about 20% (within the errors of the assay) of the biological activity exhibited at the time the pharmaceutical formulation was prepared as determined in an antigen binding assay or a potency assay, for example.

10 Herein, “biological activity” of a monoclonal antibody refers to the ability of the antibody to bind to antigen. It can further include antibody binding to antigen and resulting in a measurable biological response which can be measured *in vitro* or *in vivo*. Such activity may be antagonistic or agonistic.

15 The antibody which is formulated is essentially pure and desirably essentially homogeneous (e.g., free from contaminating proteins etc.). “Essentially pure” antibody means a composition comprising at least about 90% by weight of the antibody, based on total weight of the composition, or at least about 95% by weight. “Essentially homogeneous” antibody means a composition comprising at least about 99% by weight of antibody, based on total weight of the composition.

20 By “isotonic” is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

As used herein, “buffer” refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components.

25 Herein, a “surfactant” refers to a surface-active agent, typically a nonionic surfactant. Examples of surfactants herein include polysorbate (for example, polysorbate 20 and, polysorbate 80); poloxamer (e.g. poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, 30 or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g. lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUATTM series (Mona Industries, Inc., Paterson, N.J.); polyethyl glycol,

polypropyl glycol, and copolymers of ethylene and propylene glycol (e.g. Pluronics, PF68 etc); etc. In certain embodiments, the surfactant is polysorbate 20.

As used herein, “treatment” refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed before or during the course of clinical pathology. Desirable effects of treatment include preventing the occurrence or recurrence of a disease or a condition or symptom thereof, alleviating a condition or symptom of the disease, diminishing any direct or indirect pathological consequences of the disease, decreasing the rate of disease progression, ameliorating or palliating the disease state, and achieving remission or improved prognosis.

An “effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A “therapeutically effective amount” of a therapeutic agent may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the therapeutic agent are outweighed by the therapeutically beneficial effects.

An “individual,” “subject” or “patient” is a vertebrate. In certain embodiments, the vertebrate is a mammal. Mammals include, but are not limited to, primates (including human and non-human primates) and rodents (e.g., mice and rats). In certain embodiments, a mammal is a human.

A “medicament” is an active drug to treat a disease, disorder, and/or condition.

“Antibodies” (Abs) and “immunoglobulins” (Igs) refer to glycoproteins having similar structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which generally lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

The terms “antibody” and “immunoglobulin” are used interchangeably in the broadest sense and include monoclonal antibodies (*e.g.*, full length or intact monoclonal antibodies), polyclonal antibodies, monovalent antibodies, multivalent antibodies, multispecific antibodies (*e.g.*, bispecific antibodies so long as they exhibit the desired biological activity) and may also include certain antibody fragments (as described in greater detail herein). An antibody can be chimeric, human, humanized and/or affinity matured.

The terms “full length antibody,” “intact antibody” and “whole antibody” are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain the Fc region.

5 “Antibody fragments” comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. Examples of antibody fragments include Fab, Fab’, F(ab’)₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

10 Papain digestion of antibodies produces two identical antigen-binding fragments, called “Fab” fragments, each with a single antigen-binding site, and a residual “Fc” fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab’)₂ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

15 “Fv” is a minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. Collectively, the six CDRs of an Fv confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than
20 the entire binding site.

 The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab’ fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more
25 cysteines from the antibody hinge region. Fab’-SH is the designation herein for Fab’ in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab’)₂ antibody fragments originally were produced as pairs of Fab’ fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

30 The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible mutations, e.g., naturally occurring mutations, that may be present in minor amounts. Thus, the modifier “monoclonal” indicates the character of the antibody as not being a mixture of discrete

antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences.

- 5 For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*,
10 to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition
15 to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

- The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For
20 example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (e.g., Kohler et al., *Nature*, 256: 495 (1975); Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981)),
25 recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567), phage display technologies (see, e.g., Clackson et al., *Nature*, 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-
30 132(2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO98/24893; WO96/34096; WO96/33735; WO91/10741; Jakobovits et al., *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits et al., *Nature* 362: 255-258 (1993); Bruggemann et al., *Year in Immunol.* 7:33 (1993);

U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016; Marks et al., *Bio.Technology* 10: 779-783 (1992); Lonberg et al., *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild et al., *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996) and Lonberg and Huszar, 5 *Intern. Rev. Immunol.* 13: 65-93 (1995).

The monoclonal antibodies herein specifically include “chimeric” antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical 10 with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81:6855-9855 (1984)).

“Native antibodies” refer to naturally occurring immunoglobulin molecules with 15 varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (V_H), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, 20 from N- to C-terminus, each light chain has a variable region (V_L), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa and lambda, based on the amino acid sequence of its constant domain.

The term “variable region” or “variable domain” refers to the domain of an 25 antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (V_H and V_L, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (*See, e.g.*, Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single V_H or V_L 30 domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a V_H or V_L domain from an antibody that binds the antigen to screen a library of complementary V_L or V_H domains, respectively. *See e.g.*, Portolano *et al.*, *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

A “humanized” antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g.,
 5 CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

10 The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). A number of hypervariable region delineations are in use and are encompassed herein. The Kabat
 15 Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM hypervariable regions represent a
 20 compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The “contact” hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

	<u>Loop</u>	<u>Kabat</u>	<u>AbM</u>	<u>Chothia</u>	<u>Contact</u>
25	L1	L24-L34	L24-L34	L26-L32	L30-L36
	L2	L50-L56	L50-L56	L50-L52	L46-L55
	L3	L89-L97	L89-L97	L91-L96	L89-L96
	H1	H31-H35B	H26-H35B	H26-H32	H30-H35B (Kabat Numbering)
	H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia Numbering)
30	H2	H50-H65	H50-H58	H53-H55	H47-H58
	H3	H95-H102	H95-H102	H96-H101	H93-H101

Hypervariable regions may comprise “extended hypervariable regions” as follows: 24-36 or 24-34 (L1), 46-56 or 49-56 or 50-56 or 52-56 (L2) and 89-97 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102 or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat *et al.*, *supra* for each of these definitions.

Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas *et al. Cellular and Mol. Immunology*, 4th ed. (W. B. Saunders, Co., 2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

The “class” of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively.

An “isolated” biological molecule, such as a nucleic acid, polypeptide, or antibody, is one which has been identified and separated and/or recovered from at least one component of its natural environment.

A “subcutaneous administration device” refers to a device which is adapted or designed to administer a drug, for example a therapeutic antibody, or pharmaceutical formulation by the subcutaneous route. Exemplary subcutaneous administration devices include, but are not limited to, a needle safety device (*e.g.*, one comprising a pre-filled syringe), an injection device, including an autoinjector (*e.g.*, one comprising a pre-filled syringe), infusion pump, injector pen, needleless device, and patch delivery system. A subcutaneous administration device administers a certain volume of the pharmaceutical

formulation, for example about 0.5 mL, about 0.7 mL, about 1.0 mL, about 1.25 mL, about 1.4 mL, about 1.5 mL, about 1.75 mL, about 2.0 mL or about 5.0 mL.

A “package insert” or “label” is used to refer to instructions customarily included in commercial packages of therapeutic products or medicaments, that contain
5 information about the indications, usage, dosage, administration, contraindications, other therapeutic products to be combined with the packaged product, and/or warnings concerning the use of such therapeutic products or medicaments and the like.

As used herein, the term “gastrointestinal inflammatory disorders” refer to a group of chronic disorders that cause inflammation and/or ulceration in the mucous
10 membrane. These disorders include, for example, inflammatory bowel disease (e.g., Crohn’s disease, ulcerative colitis, indeterminate colitis and infectious colitis), mucositis (e.g., oral mucositis, gastrointestinal mucositis, nasal mucositis and proctitis), necrotizing enterocolitis, and esophagitis.

“Inflammatory Bowel Disease” or “IBD” is used interchangeably herein to refer
15 to diseases of the bowel that cause inflammation and/or ulceration and includes without limitation Crohn’s disease and ulcerative colitis.

“Crohn’s disease (CD)” or “ulcerative colitis (UC)” are chronic inflammatory bowel diseases of unknown etiology. Crohn’s disease, unlike ulcerative colitis, can affect any part of the bowel. The most prominent feature Crohn’s disease is the granular,
20 reddish-purple edematous thickening of the bowel wall. With the development of inflammation, these granulomas often lose their circumscribed borders and integrate with the surrounding tissue. Diarrhea and obstruction of the bowel are the predominant clinical features. As with ulcerative colitis, the course of Crohn’s disease may be continuous or relapsing, mild or severe, but unlike ulcerative colitis, Crohn’s disease is
25 not curable by resection of the involved segment of bowel. Most patients with Crohn’s disease require surgery at some point, but subsequent relapse is common and continuous medical treatment is usual.

Crohn’s disease may involve any part of the alimentary tract from the mouth to the anus, although typically it appears in the ileocolic, small-intestinal or colonic-
30 anorectal regions. Histopathologically, the disease manifests by discontinuous granulomatous, crypt abscesses, fissures and aphthous ulcers. The inflammatory infiltrate is mixed, consisting of lymphocytes (both T and B cells), plasma cells, macrophages, and neutrophils. There is a disproportionate increase in IgM- and IgG-secreting plasma cells, macrophages and neutrophils. Anti-inflammatory drugs

sulfasalazine and 5-aminosalicylic acid (5-ASA) are useful for treating mildly active colonic Crohn's disease and is commonly prescribed to maintain remission of the disease. Metronidazole and ciprofloxacin are similar in efficacy to sulfasalazine and appear to be particularly useful for treating perianal disease. In more severe cases, corticosteroids are effective in treating active exacerbations and can even maintain remission. Azathioprine and 6-mercaptopurine have also shown success in patients who require chronic administration of corticosteroids. It is also possible that these drugs may play a role in the long-term prophylaxis. Unfortunately, there can be a very long delay (up to six months) before onset of action in some patients.

Antidiarrheal drugs can also provide symptomatic relief in some patients. Nutritional therapy or elemental diet can improve the nutritional status of patients and induce symptomatic improvement of acute disease, but it does not induce sustained clinical remissions. Antibiotics are used in treating secondary small bowel bacterial overgrowth and in treatment of pyogenic complications.

"Ulcerative colitis (UC)" afflicts the large intestine. The course of the disease may be continuous or relapsing, mild or severe. The earliest lesion is an inflammatory infiltration with abscess formation at the base of the crypts of Lieberkühn. Coalescence of these distended and ruptured crypts tends to separate the overlying mucosa from its blood supply, leading to ulceration. 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 required for acute, severe or chronic, unremitting ulcerative colitis. The clinical features of UC are highly variable, and the onset may be insidious or abrupt, and may include diarrhea, tenesmus and relapsing rectal bleeding. With fulminant involvement of the entire colon, toxic megacolon, a life-threatening emergency, may occur. Extraintestinal manifestations include arthritis, pyoderma gangrenosum, uveitis, and erythema nodosum.

The term "serum sample" refers to any serum sample obtained from an individual. Methods for obtaining sera from mammals are well known in the art.

The term "whole blood" refers to any whole blood sample obtained from an individual. Typically, whole blood contains all of the blood components, e.g., cellular components and plasma. Methods for obtaining whole blood from mammals are well known in the art.

THERAPEUTIC AGENTS

A therapeutic agent for treating gastrointestinal inflammatory disorders (e.g., Inflammatory bowel disease) is provided herein. Inflammatory bowel disease (IBD) is a chronic gastrointestinal disease that severely affects patient quality of life and often results in the need for surgical intervention (Casellas et al., *Dig Dis.* 1999;17(4):208–18; Carter et al., *Gut.* 2004;53(Suppl 5):V1–16; Borren et al., *Nat Rev Gastroenterol Hepatol.* 2019;16(4):247–59). The predominant forms of IBD are ulcerative colitis (UC) and Crohn's disease, 2 distinct conditions that share some common symptoms and exhibit a partially overlapping etiology (Zhang and Li, *World J Gastroenterol.* 2014;20(1):91–9; Abraham and Cho, *N Engl J Med.* 2009;361(21):2066–78). Current pharmacologic therapies for IBD are not curative. In addition, many pharmacologic therapies for IBD lose efficacy over the duration of the disease and can result in systemic side effects (Abraham and Cho, *N Engl J Med.* 2009;361(21):2066–78; Rogler, *Best Pract Res Clin Gastroenterol.* 2010;24(2):157–65). Etrolizumab is an anti- β 7 integrin monoclonal antibody in development for patients with UC and Crohn's disease. Etrolizumab selectively inhibits α 4 β 7 and α E β 7 to reduce trafficking of immune cells into the gut and subsequent inflammatory effects on the gut lining (Zundler et al., *Gut.* 2019;68(9):1688–700). The efficacy and safety of etrolizumab in patients with UC was demonstrated in the phase 2 EUCALYPTUS study (Vermeire et al., *Lancet.* 2014;384(9940):309–18).

In certain embodiments, the therapeutic agent is an anti-integrin beta7 antibody or an antigen-binding fragment thereof. In certain embodiments, the anti-integrin beta7 antibody is a humanized monoclonal anti-integrin beta7 antibody. In certain such embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein: (i) the HVR-L1 comprises the amino acid sequence set forth SEQ ID NO: 1; (ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO: 2; (iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO: 3; (iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO: 4; (v) the HVR-H2 comprises the amino acid sequence set forth in SEQ ID NO: 5; and (vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO: 6 or SEQ ID NO: 7.

In certain such embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof further comprises a light chain variable region domain comprising the

amino acid sequence set forth in SEQ ID NO:8 and a heavy chain variable region domain comprising the amino acid sequence set forth in SEQ ID NO:9. In certain embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof comprises a light chain variable region domain comprising the amino acid sequence set forth in SEQ ID NO: 8 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 11 or a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 12. In certain embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO: 10 and a heavy chain variable region domain comprising the amino acid sequence set forth in SEQ ID NO: 9.

In certain such embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof further comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 11 or a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 12. In certain such embodiments, the anti-integrin beta7 antibody is etrolizumab. SEQ ID NOS: 1-12 are provided below. Anti-integrin beta7 antibodies or antigen-binding fragments thereof are further described in Intn'l Pub. No. WO2006/026759, which is incorporated herein by reference.

SEQ ID NO	Description	Sequence
1	anti-integrin beta7 antibody HVR-L1	RASESVDDLH
2	anti-integrin beta7 antibody HVR-L2	KYASQSIG
3	anti-integrin beta7 antibody HVR-L3	QQGNSLPNT
4	anti-integrin beta7 antibody HVR-H1	GFFITNNYWG
5	anti-integrin beta7 antibody HVR-H2	GYISYSGSTSYNPSLKS
6	anti-integrin beta7 antibody HVR-H3.v1	RTGSSGYFDF
7	anti-integrin beta7 antibody HVR-H3.v2	ARTGSSGYFDF
8	anti-integrin beta7 antibody VL	DIQMTQSPSSLSASVGDRVTITCRASEVD DLLHWYQQKPGKAPKLLIKYASQSIGVPS RFGSGSGTDFTLTISLQPEDFATYYCQQ GNSLPNTFGQGTKVEIKR
9	anti-integrin beta7 antibody VH	EVQLVESGGGLVQPGGSLRLSCAASGFFIT NNYWGVRQAPGKLEWVGYSYSGSTSYN PSLKSRTIS RDTSKNTFYLMNSLRAEDT AVYYCARTGSSGYFDFWGQGLVTVSS

10	anti-integrin beta7 antibody LC	DIQMTQSPSS LSASVGDRVT ITCRASESVD DLLHWYQQKPGKAPKLLIKYASQSIGVPS RFGSGSGTD FTLTISSLQPEDFATYYCQQ GNSLPNTFGQGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFN RGE
11	anti-integrin beta7 antibody HC.v1	EVQLVESGGG LVQPGGSLRLSCAASGFFIT NNYWGWVRQAPGKGLEWVGYSYSGSTSYN PSLKSRTIS RDTSKNTFYLMNSLRAEDT AVYYCARTGSSGYFDWVGQ TLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYF PEPVTWSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMEALHNHYTQKSLSLSPG
12	anti-integrin beta7 antibody HC.v2	EVQLVESGGG LVQPGGSLRLSCAASGFFIT NNYWGWVRQAPGKGLEWVGYSYSGSTSYN PSLKSRTIS RDTSKNTFYLMNSLRAEDT AVYYCARTGSSGYFDWVGQ TLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYF PEPVTWSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMEALHNHYTQKSLSLSPGK

CERTAIN MOLECULAR BIOMARKERS

[0001] In certain instances, biomarkers are quantitated in a biological sample obtained from a subject as a means of selecting subjects for treatment with a given therapeutic agent. International Pat. Nos. WO2014160753, WO2015148809, and

- 5 WO2009140684 describe methods of predicting the responsiveness of subjects having a gastrointestinal inflammatory disorder to anti-integrin beta7 antibody formulations described herein, and methods of selecting subjects having a gastrointestinal inflammatory disorder for treatment with anti-integrin beta7 antibody formulations described herein.

GENERAL TECHNIQUES FOR FORMULATIONS

Formulations comprising anti-integrin beta7 antibodies or antigen-binding fragments thereof may be prepared and analyzed using certain excipients and techniques known in the art and as further described herein. In certain embodiments, the antibody to be formulated has not been subjected to prior lyophilization and the formulation of interest herein is an aqueous formulation. In certain embodiments, the antibody is a full-length antibody. In certain embodiments, the antibody in the formulation is an antibody fragment, such as an F(ab')₂, in which case problems that may not occur for the full-length antibody (such as clipping of the antibody to Fab) may need to be addressed. The therapeutically effective amount of antibody present in the formulation is determined by taking into account the desired dose volumes and mode(s) of administration. In certain embodiments, from about 0.1 mg/mL to about 250 mg/mL, or from about 10 mg/mL to about 220 mg/mL, or from about 50 mg/mL to about 220 mg/mL, or from about 100 mg/mL to about 220 mg/mL, or from about 100 mg/mL to about 150 mg/mL, or from about 150 mg/mL to about 200 mg/mL is an exemplary antibody concentration in the formulation. In certain embodiments, the anti-integrin beta7 antibody is formulated at a concentration of 150 mg/mL.

An aqueous formulation is prepared comprising the anti-integrin beta7 antibody or an antigen-binding fragment thereof in a pH-buffered solution. The buffer can have a pH in the range from about 4.5 to about 6.5. In certain embodiments, the pH is greater than 5.0 and up to 7.0. In certain embodiments, the pH is greater than 5.5. In certain embodiments, the pH is between 5.5 and 6.1. In certain embodiments, the pH is between 5.6 and 6.1. In certain embodiments, the pH is 5.8 or about 5.8. In certain embodiments, the pH is 5.8. In certain embodiments, the pH is between 5.7 and 5.9. In certain embodiments, the pH is between 5.75 and 5.85. The pH of the presently disclosed formulation is higher than standard for an antibody formulation with similar excipient composition. Typical antibody formulations have a pH of 5.5, whereas the presented disclosed formulation has a pH of greater than 5.5, e.g., a pH of 5.8, between 5.7 and 5.9 or between 5.75 and 5.85. The higher formulation pH lowers the risk of particle formation as a result of polysorbate degradation during long term storage in a pre-filled syringe at high protein concentration. The risk of particle formation is lowered due to the increased solubility of free fatty acids at the higher pH which can result from polysorbate degradation. A pH of greater than 5.5, e.g., a pH of 5.8, balances the risk of particle formation with the chemical and physical stability of the antibody. A pH of greater than

5.5., e.g., a pH of 5.8, minimizes the rate of Asp isomerization and succinimide intermediate formation, which allows for patient convenience in combination with the device by allowing storage at ambient temperatures without substantially impacting the chemical stability of the antibody and thereby the product quality.

5 Examples of buffers that will control the pH within this range include acetate (e.g. histidine acetate, arginine acetate, sodium acetate), succinate (such as histidine succinate, arginine succinate, sodium succinate), gluconate, citrate and other organic acid buffers and combinations thereof. The buffer concentration can be from about 1 mM to about 600 mM, depending, for example, on the buffer and the desired isotonicity of the
10 formulation. In certain embodiments, the buffer comprises histidine. The presence of histidine in the formulation can greatly reduce the rate of high molecular weight species (HMWS) formation in the presence of zinc. The concentration of the histidine in the formulation can be between about 5 mM and about 40 mM, between about 5 mM and about 30 mM, between about 10 mM and about 40 mM, between about 10 mM and
15 about 30 mM, between about 15 mM and about 25 mM, between about 10 mM and about 20 mM, or between about 15 mM and about 20 mM. In certain embodiments, the concentration of the histidine in the formulation is about 20 mM.

In certain embodiments, the buffer is 20 mM histidine, pH 5.8.

In certain embodiments, the formulation comprises arginine succinate. In certain
20 embodiments, the concentration of the arginine succinate in the formulation is from about 20 mM to 300 mM. In certain embodiments, the concentration of the arginine succinate in the formulation is from about 100 mM to 300 mM, from about 100 mM to about 200 mM, from about 150 mM to about 300 mM, from about 200 mM to about 300 mM, from about 100 mM to about 250 mM, from about 150 mM to about 250 mM, or
25 from about 150 mM to about 200 mM. In certain embodiments, the concentration of the arginine succinate in the formulation is about 200 mM. The high arginine concentration, and high conductivity formulation shields charge on the antibody and prevents shifts in pH. In addition, arginine can impact the viscosity of the formulation, e.g., formulation viscosity is decreased by the addition of arginine to the formulation.

30 The formulation has a viscosity of less than about 20 centipoise (cP) at 25°C. In certain embodiments, the viscosity of the formulation is between about 1 cP and about 20 cP at 25°C, between about 5 cP and about 20 cP at 25°C, between about 5 cP and about 15 cP at 25°C, between about 1 cP and about 10 cP at 25°C, or between about 5 cP and

about 10 cP at 25°C. In certain embodiments, the viscosity of the formulation is about 7 cP at 25°C.

In certain embodiments, the antibody formulation comprises a surfactant.

Exemplary surfactants include nonionic surfactants. Suitable non-ionic surfactants

5 include polysorbates (20, 40, 60, 65, 80, etc.), poloxamers (184, 188, etc.), Pluronic polyols, Triton®, polyoxyethylene sorbitan monoethers (Tween®-20, Tween®-80, etc.), laurmacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl
10 sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride. In certain embodiments, the surfactant is polysorbate 20.

Non-ionic surfactants can help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits
15 the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody.

The amount of surfactant added is such that it reduces aggregation of the formulated antibody and/or minimizes the formation of particulates in the formulation and/or reduces adsorption. For example, the surfactant may be present in the formulation
20 in an amount of greater than 0.005% weight/volume (w/v). In certain embodiments, the concentration of the surfactant in the formulation is greater than 0.005% w/v and up to about 1% w/v. The concentration of the surfactant in the formulation can be between about 0.005% and about 0.5% w/v, between about 0.02% w/v and about 0.5% w/v, between about 0.03% w/v and about 0.5% w/v, between 0.03% w/v and 0.1% w/v. In
25 certain embodiments, concentration of the surfactant in the formulation is 0.04% w/v. In certain embodiments, the surfactant is polysorbate 20 present in the formulation in an amount of 0.04% w/v. The typical concentration of polysorbate 20 for an antibody formulation is 0.02% (w/v). The presently disclosed formulation comprises 0.04% w/v Polysorbate 20. The higher concentration of polysorbate 20 helps solubilize free fatty
30 acids, which can be generated as a result of polysorbate degradation, thereby lowering the risk of forming particles.

In certain embodiment, the formulation contains the above-identified agents (e.g., antibody, buffer, and surfactant) and is essentially free of one or more preservatives,

such as benzyl alcohol, phenol, m-cresol, chlorobutanol and benzethonium Cl. In certain embodiments, the formulation does not comprise a preservative. In certain embodiments, a preservative may be included in the formulation, particularly where the formulation is a multidose formulation. The concentration of preservative may be in the
5 range from about 0.1% to about 2%, or from about 0.5% to about 1%. One or more other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) may be included in the formulation provided that they do not adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are
10 nontoxic to recipients at the dosages and concentrations employed and include; additional buffering agents; co-solvents; anti-oxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (e.g. Zn-protein complexes); biodegradable polymers such as polyesters; and/or salt-forming counterions.

While the various descriptions of chelators herein often focus on EDTA, it will
15 be appreciated that other metal ion chelators are also encompassed within the invention. Metal ion chelators are well known by those of skill in the art and include, but are not necessarily limited to aminopolycarboxylates, EDTA (ethylenediaminetetraacetic acid), EGTA (ethylene glycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid), NTA (nitrilotriacetic acid), EDDS (ethylene diamine disuccinate), PDTA (1,3-
20 propylenediaminetetraacetic acid), DTPA (diethylenetriaminepentaacetic acid), ADA (beta-alaninediacetic acid), MGCA (methylglycinediacetic acid), etc. Additionally, some embodiments herein comprise phosphonates/phosphonic acid chelators.

Tonicity agents, sometimes known as "stabilizers" are present to adjust or maintain the tonicity of a liquid composition. When used with large, charged
25 biomolecules such as proteins and antibodies, they are often termed "stabilizers" because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter and intra-molecular interactions. Tonicity agents can be present in any amount between 0.1% to 25% by weight, or 1 to 5%, taking into account the relative amounts of the other ingredients. Tonicity agents include polyhydric sugar alcohols,
30 trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

Additional stabilizers include a broad range of excipients which range in function from bulking agents to solubility enhancers, to agents preventing denaturation or adherence to the container wall. Stabilizers can be present in the range from 0.1 to

10,000 parts per weight active protein or antibody. Typical stabilizers include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, methionine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinisitol, myoinisitol, galactose, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, a-monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (e.g., xylose, mannose, fructose, glucose; disaccharides (e.g., lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

Various analytical techniques for measuring protein stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 2990 (1993), for example. Stability can be measured at a selected temperature for a selected time period. In certain embodiments, the formulation is stable at about 40°C for at least about 1 week. In certain embodiments, the formulation is stable at about 5°C for at least about 12 months, and/or or stable at about 5°C for at least about 18 months, and/or or stable at about 5°C for at least about 2 years, and/or or stable at about 5°C for at least about 3 years, and/or or stable at about 5°C for at least about 4 years, and/or or stable at about 5°C for at least about 5 years. In certain embodiments, the formulation is stable at about -20°C for at least 2 years, and/or stable at about -20°C for at least 4 years, and/or stable at about -20°C for at least about 5 years, and/or stable at about -20°C for at least about 6 years, and/or stable at about -20°C for at least about 7 years. In certain embodiments, the formulation is stable at about 25°C for at least about 1 week, and/or stable at about 25°C for at least about 2 weeks, or stable at about 25°C for at least about 4 weeks. In certain embodiments, the formulation is stable following freezing (to, e.g., -70°C) and thawing of the formulation, for example following 1, 2, 3, 4, or 5 cycles of freezing and thawing. Stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using cation exchange

chromatography, image capillary isoelectric focusing (icIEF) or capillary zone electrophoresis; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis; SDS-PAGE analysis to compare reduced and intact antibody; peptide map (for example tryptic or LYS-C) analysis; evaluating biological activity or antigen binding function of the antibody; etc. Instability may involve any one or more of: aggregation, deamidation (e.g. Asn deamidation), oxidation (e.g. Met oxidation), isomerization (e.g. Asp isomerization), clipping/hydrolysis/fragmentation (e.g. hinge region fragmentation), succinimide formation, unpaired cysteine(s), N-terminal extension, C-terminal processing, glycosylation differences, etc.

10 The formulations to be used for *in vivo* administration should be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to, or following, preparation of the formulation.

 In certain embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof is administered using, for example, a self-inject device, autoinjector device, or other device designed for self-administration. In certain embodiments, the anti-integrin beta7 antibody or antigen-binding fragment thereof is administered using a subcutaneous administration device. Various self-inject devices and subcutaneous administration devices, including autoinjector devices, are known in the art and are commercially available. Exemplary devices include, but are not limited to, prefilled syringes (such as BD HYPAK SCF®, BD NEOPAK™, READYFILL™, and STERIFILL SCF™ from Becton Dickinson; CLEARSHOT™ copolymer prefilled syringes from Baxter; and Daikyo Seiko CRYSTAL ZENITH® prefilled syringes available from West Pharmaceutical Services); disposable pen injection devices such as BD Pen from Becton Dickinson; ultra-sharp and microneedle devices (such as INJECT-EASE™ and microinfuser devices from Becton Dickinson; and H-PATCH™ available from Valeritas) as well as needle-free injection devices (such as BIOJECTOR® and IJECT® available from Bioject; and SOF-SERTER® and patch devices available from Medtronic). Certain embodiments of subcutaneous administration devices are described further herein. Co-formulations or co-administrations with such self-inject devices or subcutaneous administration devices of an anti-integrin beta7 antibody or an antigen-binding fragment thereof with at least a second therapeutic compound are envisioned.

RECOMBINANT METHODS

Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In certain embodiments, a nucleic acid

molecule encoding an anti-integrin beta7 antibody or an antigen-binding fragment thereof described herein is provided. Such nucleic acid molecule may encode an amino acid sequence comprising the V_L and/or an amino acid sequence comprising the V_H of the antibody (e.g., the light and/or heavy chains of the antibody). In certain

5 embodiments, one or more vectors (e.g., expression vectors) comprising such nucleic acid molecules are provided. In certain embodiments, a host cell comprising such nucleic acid molecule is provided. In certain embodiments, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid molecule that encodes an amino acid sequence comprising the V_L of the antibody and an amino acid sequence

10 comprising the V_H of the antibody, or (2) a first vector comprising a nucleic acid molecule that encodes an amino acid sequence comprising the V_L of the antibody and a second vector comprising a nucleic acid molecule that encodes an amino acid sequence comprising the V_H of the antibody. In certain embodiments, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., YO, NSO, Sp20 cell).

15 In certain embodiments, a method of making an anti-integrin beta7 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid molecule encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

20 For recombinant production of an anti-integrin beta7 antibody, nucleic acid molecules encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid molecules may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes

25 encoding the heavy and light chains of the antibody).

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g.,

30 U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been “humanized,” resulting in the production of an antibody with a partially or fully human glycosylation pattern.

- 5 See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified
10 which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures can also be utilized as hosts. See, e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIESTM technology for producing antibodies in transgenic plants).

- 15 Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4
20 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC
25 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR-CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as YO, NSO and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana
30 Press, Totowa, NJ), pp. 255-268 (2003).

ASSAYS

Anti-integrin beta7 antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

- 5 The etrolizumab potency assay measures the ability of etrolizumab to inhibit RPMI8866 B cell binding to MAdCAM. In this assay, MAdCAM was coated onto a 96-well microtiter plate. Following overnight incubation, etrolizumab standards, controls, and samples were added to the plate, along with a fixed amount of cells. The plate was incubated at 37°C in a humidified incubator to allow binding of the cells to the
- 10 MAdCAM. A wash step was performed to remove non-adherent cells, and the remaining live cells were quantified by adding the redox dye alamar Blue, which is blue and non-fluorescent in its oxidized state but is reduced by the intracellular environment into a pink form that is highly fluorescent. Thus, changes in color and fluorescence were proportional to the number of bound viable cells. The results were expressed in RFU
- 15 and plotted against etrolizumab concentration. Parallel curve analysis was used to estimate the activity of the etrolizumab sample(s) relative to the Reference Material.

Potency Assay

ARTICLES OF MANUFACTURE AND KITS

- 20 An article of manufacture is provided which comprises the formulation and provides instructions for its use. The article of manufacture comprises a container.

- In certain embodiments, an article of manufacture comprising a subcutaneous administration device is provided which delivers to a subject a flat dose of an anti-integrin beta7 antibody or an antigen-binding fragment thereof, wherein the flat dose is for example, but not limited to, 105 mg, or 210 mg. In certain embodiments, the anti-
- 25 integrin beta7 antibody is etrolizumab. The anti-integrin beta7 antibody or antigen-binding fragment thereof in the subcutaneous administration device is formulated in a buffer, for example, histidine pH 5.8, and other excipients, for example, polysorbate and arginine succinate, such that it is provided in a stable pharmaceutical formulation.

- In certain embodiments, the subcutaneous administration device is a prefilled
- 30 syringe comprising a glass barrel with needle and optionally, a needle shield and also optionally, a needle shield device. In certain embodiments, the volume of the formulation contained in the syringe is between about 0.1 mL and about 2 mL, between about 0.1 mL and about 2 mL, between about 0.5 mL and about 2 mL, or between about

1 mL and about 2 mL. In certain embodiments, the volume of the formulation contained in the syringe is between about 0.5 mL and about 2 mL. In certain embodiments, the volume of the formulation contained in the syringe is about 0.5 mL, about 0.7 mL, about 1 mL, about 1.4 mL, about 1.5 mL, or about 2.0 mL. In certain embodiments, the volume of the formulation contained in the syringe is about 0.7 mL. In certain embodiments, the volume of the formulation contained in the syringe is about 0.75 mL. In certain embodiments, the volume of the formulation contained in the syringe is about 1 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is between about 0.5 mL and about 1.0 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is between about 1.0 mL and about 1.5 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is about 1.4 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is about 1.5 mL. In certain embodiments, the volume of the formulation contained in the prefilled syringe is about 1.45 mL.

15 In certain embodiments, the needle is a staked-in needle comprising a 3-bevel tip or a 5-bevel tip. In certain embodiments, the needle is between 25 gauge (G) and 30G. In certain embodiments, the needle is between 1/2 inch long and 5/8 inch long. In certain embodiments, the subcutaneous administration device comprises a prefilled 1.0 mL low tungsten borosilicate glass (type I) syringe and a stainless steel 5-bevel 27G 1/2
20 inch long thin-wall staked-in needle. In certain embodiments, the subcutaneous administration device comprises a rigid needle shield. In certain embodiments, the rigid needle shield comprises a rubber formulation having low zinc content and comprises a rigid polypropylene shield. In certain embodiments, the rubber plunger stopper comprises Daikyo 777-7 rubber and FluroTec® ethylene-tetrafluoroethylene (ETFE)
25 coating (West Pharmaceutical Services, Inc.). In certain embodiments, the subcutaneous administration device comprises a needle safety device. Exemplary needle safety devices include, but are not limited to, Ultrasafe Passive® Needle Guard X100L (Becton Dickinson and Company) and Rexam Safe n Sound™ (Rexam).

In certain embodiments, the injection device is a prefilled syringe. Non-limiting
30 examples of prefilled syringes include BD HYPAK SCF®, READYFILL™, and STERIFILL SCF™ from Becton Dickinson; CLEARSHOT™ copolymer prefilled syringes from Baxter; and Daikyo Seiko CRYSTAL ZENITH® prefilled syringes available from West Pharmaceutical Services. In certain embodiments, the prefilled syringe comprises silicone oil. The etrolizumab prefilled syringe was developed with

optimal levels of silicone oil in order to ensure low injection forces while maintaining a low risk of particle formation. Free fatty acids from degraded polysorbate partition to silicone oil to a greater or lesser extent depending on the pH and the Polysorbate concentration. Hence, the specific combination of the formulation excipients at the target pH and the target levels of silicone oil in combination are uniquely well suited to long term storage of a high concentration antibody formulation stored in a prefilled syringe. In certain embodiments, the amount of silicone oil in the prefilled syringe is not greater than about 1 mg. In certain embodiments, the amount of silicone oil in the prefilled syringe is between about 0.1 mg and about 1 mg. In certain embodiments, the amount of silicone oil in the prefilled syringe is between about 0.2 mg and about 0.6 mg. In certain embodiments, the amount of silicone oil in the prefilled syringe is between about 0.5 mg and 0.9 mg.

The prefilled syringe may have any suitable syringe capacity. In certain embodiments, the prefilled syringe has a syringe capacity of between about 0.5 mL and about 10 mL, between about 0.5 mL and about 5 mL, between about 0.5 mL and about 2.5 mL, between about 1 mL and about 5 mL, or between about 1 mL and about 2.5 mL. In certain embodiments, the prefilled syringe has a syringe capacity of 1 mL. In certain embodiments, the prefilled syringe has a syringe capacity of 2.25 mL.

In certain embodiments, the injection device is an autoinjector, e.g., autoinjectors disclosed in U.S. Pat. Nos. 2014/0148763 and 2014/0114247, which are incorporated by reference herein. In certain embodiments, the autoinjector is a single-use autoinjector. In certain embodiments, the autoinjector is based on Rotaject[®] technology (e.g., accessible at <https://www.shl.group/products-and-services/rotaject-technology-auto-injector/>).

Additional devices suitable for subcutaneous delivery include for example, but not limited to, an injection device such as INJECT-EASE[™] and GENJECT[™] devices; an infusion pump such as ACCU-CHECK[™]; an injector pen such as BD Vystra[™] from Becton Dickinson, a needleless device such as MEDDCTOR[™] and BIOJECTOR[™] and IJECT[™], and a subcutaneous patch delivery system such as BD Libertas[™], H-PATCH[™] available from Valeritas, and SOF-SERTER[™].

Kits will typically comprise the container described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with

instructions for use. A label may be present on the container to indicate that the composition is used for a specific therapy.

EXAMPLES

5 The following are examples of the formulations and methods of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1

10 Important aspects of the optimization of the etrolizumab drug product formulation and configuration are minimizing viscosity to minimize injection force, maximizing the accuracy of the injection volume and optimizing compatibility with device components. Balancing the optimal formulation excipients to sufficiently protect the antibody from physical and chemical degradation, while maintaining biological activity (potency) and achieving high concentration of antibody while maintaining low
15 viscosity is important. These aspects of the formulation must be balanced in concert with the prefilled syringe configuration and specifications such as silicone oil concentration, syringe materials of construction, barrel width, and needle wall thickness, which allow for lower injection force, shorter injection time and less pain as the result of injection.

20 In the present example, an etrolizumab formulation comprising 150 mg/ml etrolizumab, 20 mM histidine, 200 mM arginine succinate, and 0.04% w/v of polysorbate 20 (PS20) at pH 5.8 was prepared. It was shown that this etrolizumab formulation was unexpectedly well suited for long-term storage at high concentration in a pre-filled syringe. Furthermore, when paired with prefilled syringes, this formulation
25 minimized the risk of subvisible and visible particle formation upon long-term storage, which allow for extended shelf-life and patient-convenience. Impacts of the formulation pH, arginine, succinate, polysorbate 20, antibody concentration, and histidine on the viscosity and stability (e.g., aggregate formation, charge variants, etc.) of an etrolizumab formulation were investigated.

pH and polysorbate 20

30 The impacts of the polysorbate 20 and the pH of the solution (formulation buffer) on the solubility of lauric acid, myristic acid, and palmitic acid were investigated. As shown in Figure 1, increasing levels of polysorbate 20 increases the solubility of lauric acid, myristic acid, and palmitic acid (which can be generated as a result of polysorbate

degradation) thereby lowering the risk of forming particles. The results shown in Figure 1 also suggest that increasing pH increases the solubility of free fatty acids. pH 5.8 is an optimal formulation pH which balances the risk of particle formation with the chemical and physical stability of the antibody. A formulation of this pH minimizes the rate of aspartic acid (Asp) isomerization and succinimide intermediate formation, which allows for patient convenience in combination with the device by allowing storage at ambient temperatures without substantially impacting the chemical stability of the antibody and thereby the product quality. Furthermore, the risk of particle formation is lowered due to the increased solubility of free fatty acids at the higher pH which can result from polysorbate degradation.

Arginine

Low viscosity is desired, as low viscosity can reduce injection force and ensure accuracy of injection volume. Use of arginine hydrochloride or arginine succinate in antibody formulations has been described previously. See, e.g., U.S. Patent No. 8,142,776, and International Patent Application Publication Nos. WO2006065746 and WO2010102241. The impact of the arginine succinate on the viscosity of the formulation was investigated. First, the viscosity of an etrolizumab formulation with arginine succinate was compared to the viscosity of etrolizumab formulation absent arginine succinate, and the results are shown in Figure 2. As shown in Figure 2, solution viscosity was decreased significantly by the addition of arginine succinate to the formulation. Next, the inventors investigated whether arginine, in this case, 200 mM arginine succinate, can impact the viscosity of an etrolizumab formulation of different antibody concentration. The viscosities of an etrolizumab formulation comprising 100 mg/mL, 150 mg/mL, 180 mg/mL, 200 mg/mL, and 220 mg/mL were measured, and the results are shown in Figure 3. As shown in Figure 3, with arginine succinate (e.g., 200 mM arginine succinate), formulations with antibody concentrations as high as 200 mg/mL have viscosities suitable for pre-filled syringe (PFS) administration.

Silicone oil

Because prefilled syringe drug products typically contain silicone oil and because it is known that silicone oil may cause protein aggregation and/or particle formation over time, the inventors investigated the effect of silicone oil on etrolizumab formulations.

To test the effect of silicone oil, the interior of pre-filled syringe glass barrels was sprayed with silicone oil before filling with the etrolizumab formulation. To assess

protein aggregation, high molecular weight species (HMWS) of etrolizumab were determined using size exclusion chromatography (SEC) for the determination of the aggregation and oligomeric state of antibodies including HMWS in the etrolizumab formulation. SEC was conducted on a Agilent high performance liquid chromatography (HPLC) system with a Tosoh TSKgel column and a mobile phase consisting of potassium phosphate and potassium chloride at a flowrate of 0.5 mL/min and quantified by UV absorbance and peak area integration. Briefly, the effect of different amounts of silicone oil on the formation of HMWS was determined in etrolizumab formulations comprising 150 mg/mL etrolizumab or 180 mg/mL etrolizumab. As shown in Figure 4, the increased levels of silicone oil did not impact the physical stability of all tested etrolizumab formulations under various conditions.

Hence, the specific combination of the formulation excipients at the target pH and in the presence of silicone oil are uniquely well suited to long term storage of a high concentration antibody formulation stored in a prefilled syringe. This finding is supported by about 6 years (e.g., 74 months) of long-term stability of etrolizumab in prefilled syringes without the observation of visible particles.

Succinate and Histidine

The presently disclosed formulations have the advantage to protect the antibody from additional stresses which can results from the storage in a prefilled syringe. Leachables from the prefilled syringe can lead to chemical and physical degradation of the antibody. For example, zinc and tungsten can contribute to metal-catalyzed degradation. During the syringe manufacturing process, a hot tungsten pin is inserted into the glass barrel to make the hole for needle insertion. This process can leave residual tungsten particles in the syringe barrel, which can interact with the drug solution causing the formation of aggregates. Tungsten can induce protein aggregation and formation of proteinaceous particles. Protein oxidation can be induced by tungsten as well leading to protein aggregation. Succinic acid protected from zinc-based metal-catalyzed degradation. Further, the presently disclosed formulation was not susceptible to tungsten-spiking mediated aggregation.

To determine the impact of tungsten exposure on the physical stability an etrolizumab formulation, the percentages of HMWS were determined after exposure to different quantities of tungsten over time. As shown in Figure 5, tungsten did not impact the product quality of etrolizumab.

Both the plunger stopper and the needle shield may be composed of rubber material which may leach zinc into the formulation. It is known that zinc may complex with protein leading to protein aggregates (e.g., HMWS) and increased viscosity of antibody formulations. The effect of zinc on the viscosity of etrolizumab formulations at varying protein concentrations was investigated. As shown in Figure 6, there was no difference in viscosity between formulations lacking zinc and formulations containing 10 mM zinc when the concentration of etrolizumab was 150 mg/mL or less in formulations comprising 20 mM histidine, 200 mM arginine succinate at pH 5.8. Zinc increased the viscosity at etrolizumab concentrations higher than 150 mg/mL. In addition, Figure 7 shows the formation of protein aggregates in formulations comprising 150 mg/mL etrolizumab and 50 mM zinc. Figure 8 shows that the addition of zinc increases the percentage of HMWS and that the increase in percentage of HMWS is higher at 50 mg/mL etrolizumab compared to 10 mg/mL etrolizumab.

The inventors then tested whether histidine can reduce the formation of HMWS. As shown in Figure 9, the presence of histidine in the formulation greatly reduced the rate of HMWS formation in presence of zinc. Furthermore, as shown in Figure 10, succinate also suppressed the interaction between etrolizumab and zinc to form HMWS. Next, the impact of varying concentrations of histidine and succinate on the HMWS formation was studied. As shown in Figure 11, the combination of histidine and succinate minimized the HMWS formation in the presence of zinc.

Drug Substance and Drug Substance Stability

The stability of etrolizumab drug substance (DS) formulated at 150 mg/mL in 20mM histidine, pH 5.8, 200mM arginine succinate, 0.04% polysorbate 20 was evaluated in at 30°C, 5°C, and -20°C. The stability was also evaluated up to five freeze/thaw cycles to support at-scale storage and handling. No changes in the chemical, physical, or bioactivity properties were observed after five freeze/thaw cycles or after storage of the DS at -20°C for seven years (84 months). After storage at 5°C for six months, there was no change observed by all assays except ion exchange chromatography. After storage at 30°C for 14 days, degradation was measured by nonreduced capillary electrophoresis, ion exchange, and size exclusion chromatography.

Deamidation and isomerization are degradation routes that can occur on stability. Ion exchange chromatography (IEC) was used to monitor chemical degradation and quantitates acidic variants, basic variants, and main peak. No change was observed in main peak, acidic variants, or basic variants after five freeze/thaw cycles or after 84

months of storage at -20°C. After six months of storage at 5°C, a 1.7% loss of main peak with concomitant 1.0% and 0.8% increases in acidic and basic variants, respectively, was observed. After 14 days of storage at 30°C, there was a 4.5% loss of main peak with concomitant 2.1% and 2.4% increases in acidic and basic variants, respectively.

- 5 Qualitatively, no change in ion exchange profile or new peaks were observed.

After 14 days of storage at 30°C, the following changes were observed: by non-reduced capillary electrophoresis-SDS, 1.0% loss of main peak observed with concomitant 0.82% and 0.14% increases in pre-peaks and post-peaks respectively; by IEC, there was a 4.5% loss of main peak with concomitant 2.1% and 2.4% increases in
10 acidic and basic variants, respectively; by size exclusion chromatography (SEC), increases of .28% and .09% are observed in HMWS and LMWS, respectively.

The stability of etrolizumab drug product formulated at 150 mg/mL in 20mM histidine, pH 5.8, 200mM arginine succinate, 0.04% polysorbate 20 in a 105mg (0.7 ml) prefilled syringe configuration was investigated. Etrolizumab showed no change in
15 product quality after 60 months at 5°C, as assessed by clarity, opalescence, and coloration (COC), pH, protein concentration, size exclusion chromatography (SEC), and potency. There was a 7.7% loss of main peak by ion exchange chromatography (IEC) with concomitant increases of 4.2% and 3.4% acidic and basic variants, respectively, and a 1.0% loss of main peak by CE-SDS non-reduced. After storage at 25°C for 3 months
20 there was: no change in clarity, opalescence, and coloration (COC), pH, protein concentration, or potency; a loss of 0.4% main peak by size exclusion chromatography with 0.2% increases in both high molecular weight and low molecular weight species; a 1.3% loss of main peak by CE-SDS; a 9.8% loss of main peak by ion exchange chromatography with a 5.1% increase in acidic variants and a 4.6% increase in basic
25 variants. Etrolizumab drug product stored at 5°C for 60 months was practically free of visible particles.

Conclusion

Unique aspects of the specific excipient combination of the etrolizumab formulation can protect the antibody from additional stresses which can result from the
30 storage of an antibody in a prefilled syringe, e.g., histidine and succinate can reduce HMWS formation by zinc and the antibody. Typical antibody formulations comprise polysorbate 20 at 0.02% w/v and typical antibody formulations are at pH 5.5. The higher than typical polysorbate 20 concentration (e.g., 0.04% w/v) and higher than typical pH (e.g., pH 5.8) can solubilize free fatty acids, thereby lowering the risk of particle

formation as a result of polysorbate degradation, e.g., during long term storage in a pre-filled syringe. The excipient combination of the etrolizumab formulation is especially effective at controlling pH during Tangential Flow Filtration (TFF) at large scale manufacturing. The high arginine concentration and high conductivity formulation shields charge on the antibody and prevents shifts in pH. The histidine concentration effectively buffers the formulation at the target pH. This is important to ensure robust control of the pH during manufacturing and allow for a narrow pH range of the formulation to enable this optimal configuration. The formulation also maintained chemical and physical stability and maintained potency over various time periods and at various temperatures as described above.

Example 2 - Clinical Strategies and Initial Outcomes in Support of an Etrolizumab Autoinjector in Healthy Volunteers

Summary

Etrolizumab is a novel, dual-action, anti- β 7 integrin antibody in development for patients with moderate to severe ulcerative colitis or Crohn's disease. Phase 3 studies utilize a prefilled syringe (PFS) for etrolizumab administration. In parallel, an autoinjector (AI) is being developed in order to increase delivery options for patients. This Example describe the overall development strategy and detail the first-in-human study of this developmental AI.

This open-label study of healthy volunteers (HVs) evaluated the tolerability and usability of the etrolizumab AI under development. The primary end point was the proportion of participants with greater than mild pain following injection. Adverse events (AEs) and usage errors were also assessed. Results were reported by injection site (thigh vs abdomen) and needle training (experienced vs naïve). Pharmacokinetic (PK) variability between participants was included as an exploratory end point.

Thirty participants completed the study. A total of 97% of participants never experienced greater than mild pain during the study; 50% did not experience any pain. Three usage errors were observed, 1 of which resulted in a partial dose delivery of etrolizumab. No patterns of usage errors were observed. Mild injection-site reactions (ISRs) were reported; all resolved by the end of the study. Participants injecting into the abdomen reported more ISRs than those injecting into the thigh; needle training did not appear to influence incidence or severity of AEs.

Results from this first-in-human study demonstrate that single injections of etrolizumab 105 mg using an AI are well tolerated in HVs, with transient, mild pain and

minimal usage errors. Results from this study also informed the design of a subsequent PK comparability study comparing the PFS and AI. Overall, the availability of an AI may provide an attractive option for patients desiring a convenient, easy to use delivery mechanism for etrolizumab.

5 Introduction

Etrolizumab is being evaluated in an extensive clinical program of phase 3 studies in patients with moderate to severe UC and Crohn's disease (Etro Studies. The Etro Studies: Explore Innovation: Contribute to Science. Genentech; 2019. Accessed July 26, 2019), where etrolizumab is administered once per month via subcutaneous (SC) injection using a prefilled syringe (PFS) with a needle safety device (NSD).

Single-use, prefilled autoinjectors (AIs) have many potential advantages over PFS-NSDs; most notably, their ability to keep the needle out of sight of the user at all times during injection. Exemplary PFS and AI are shown in Figure 12. AIs also offer increased convenience, ease of use, reduced risk of dosage error, and improved patient comfort. Studies have consistently shown that many patients who self-administer prefer an AI over a syringe-based device (Kivitz et al., *Clin Ther.* 2006;28(10):1619–29; Kivitz and Segurado, *Expert Rev Med Devices.* 2007;4(2):109–16; Borrás-Blasco et al., *Expert Opin Biol Ther.* 2010;10(3):301–7; Vermeire et al., *Patient Prefer Adherence.* 2018;12:1193–202). For example, a recent study of golimumab in patients with UC demonstrated that a majority of patients preferred administration with an AI versus a PFS, citing increased ease of use and reduced discomfort with injection (Vermeire et al., *Patient Prefer Adherence.* 2018;12:1193–202).

The AI currently under development consists of an automated delivery system encasing the same PFS used in the phase 3 studies (*see* Figure 13). The drug product contained in both the AI and the PFS-NSD consists of a liquid formulation of 105 mg of etrolizumab solution (0.7 mL, nominal volume of 150 mg/mL) for single-dose administration. The entire dose is typically administered in about 2 seconds.

The AI includes many features aimed to improve the patient experience and increase patient comfort with self-administration. The automated drug delivery system is activated by lightly pressing the device onto skin perpendicularly. Once activated, the AI automatically inserts the needle and dispenses the syringe contents upon activation. Once injection is complete, a needle cover extends and locks over the needle, keeping the needle out of view at all times during injection and protecting the user and others from accidental contact with the used needle. The AI also incorporates both visual and

auditory mechanisms designed to assist users with self-injection; a visible spinning top and an audible clicking sound both indicate whether drug administration is ongoing or completed. In addition, a visible plunger rod moves across the viewing window while the injection is in progress.

5 Here, the overall development strategy for a novel AI for etrolizumab, and findings from a first-in-human study of this device are shown. The primary objectives of this study (NCT02629744) were to evaluate the safety and tolerability of etrolizumab administered by the AI and primarily injection site pain following self-injection with the AI and to document critical use errors. In addition, the inventors evaluated this relatively
10 unique study design, which combined usage error assessments (traditionally conducted as simulated studies) with a tolerability, safety, and exploratory PK study.

Methods

Study Design and Procedures. This first-in-human AI tolerability study was an open-label, single-arm study in healthy volunteers evaluating pain, safety, and usability
15 of an AI when self-administered subcutaneously. Participants were assigned (1:1) into 2 groups. In order to simulate prior experience of self-injection, 1 group (“needle-experienced”) received training before self-injection with the AI; the other group (“needle-naïve”) did not. Training involved simulated needle experience by self-injection with placebo. Before etrolizumab administration, all participants (irrespective of needle
20 experience group) received an instructions for use (IFU) leaflet regarding the AI for review before self-injection. Subjects were randomly assigned to administer study drug to their abdomen or anterior thigh. All participants self-administered a single SC dose of etrolizumab on day 1 of the study in a simulated home setting (*see* Figure 14). Participants were monitored during self-injection, discharged at study day 3, and
25 returned for follow-up visits on study days 8, 29, 43, 57, and 85 (study completion).

Participants. Eligible participants were to be between the ages of 18 and 65 years, have a body mass index (BMI) of between 18.0 and 32.0 kg/m² (inclusive), and be in good health with no significant medical history or laboratory test abnormalities. Both men and women were enrolled, with the target of 55% to 60% male participants to
30 mimic the sex distribution of patients with IBD. Participants with any prior use of anti-integrin therapies (including etrolizumab) or immunosuppressive drugs were excluded, as were participants with a recent history of corticosteroid use. Participants with a history of tuberculosis were also excluded.

Procedures. Participants assigned to the needle-experienced group received training (simulated self-injection experience) 5 and 7 days before etrolizumab injection. During training, participants were instructed by a healthcare professional on the use of a needle and syringe. Following this instruction, participants practiced self-injection with a placebo solution 3 times using a needle and syringe. Needle-experienced participants deemed to be suitable by the healthcare professional (based on their interactions with syringes) progressed through the study. Both needle-experienced and needle-naïve participants were randomized, stratified by sex and needle experience, to inject into either the abdomen or anterior thigh. On study day 1, participants self-administered a single SC dose of etrolizumab 105 mg into their abdomen or anterior thigh using the AI. Participants were assessed for operational difficulties and usage errors and reported pain during and immediately following the injection. One serum sample was taken for exploratory pharmacokinetic assessment 7 days following the SC injection (study day 8).

Approaches for Assessing Pain. Pain was assessed via 2 independent methods, both of which were administered by study site personnel. The 7-point categorical Verbal Descriptive Scale (VDS-7) was the primary measure of pain for this study. During VDS-7 administration, patients were asked to choose the number from 1 to 7 that best represents the pain associated with the injection (scale as follows: 1 = no pain, 2 = very mild pain, 3 = mild pain, 4 = not very severe pain, 5 = quite severe pain, 6 = very severe pain, 7 = almost unbearable pain). As a confirmatory assessment, pain was also assessed via a 100-point continuous visual analog scale (VAS). For the VAS, patients were asked to mark a line on a horizontal 100 mm scale that best represents their pain (scale as follows: 0 mm = no pain, 100 mm = worst possible pain). Study personnel then measured the distance between the 0 mm point and the patient's mark to determine their VAS score.

Outcomes. The primary end point was the proportion of participants with greater than mild pain (VDS-7 > 3) immediately following injection. To meet the primary end point, the upper bound of the 2-sided 95% confidence interval (CI) around the proportion of participants experiencing greater than mild pain immediately following injection must not exceed 30%. Secondary end points included the proportion of participants experiencing greater than mild pain at 5, 10, 20, 60, and 240 minutes (4 hours) following injection, and the proportion of participants in each VDS-7 category over time. Tolerability was assessed intensively in this study via active monitoring for injection site reactions (ISRs) on study day 1 at 5, 60, and 240 minutes after injection, and on study

days 2, 8, 43, and 85. To identify ISRs, a local injection site symptom assessment (LISSA) was performed to assess for burning, itching, bruising, redness, and/or formation of hives, and the size of the reaction if present. All ISRs were categorized and reported as an adverse event (AE) or serious AE as appropriate. Usage errors and operational difficulties during use of the AI were documented. In addition, participants' knowledge of the IFU and their overall opinions of the AI experience were collected. Safety was assessed via AE monitoring, laboratory assessment, vital signs, physical examinations, electrocardiograms (ECGs), and immunogenicity. For this study, no formal statistical testing was planned. Determination of PK variability on a single time point of study day 8 following self-injection was assessed as an exploratory end point.

Results

Thirty healthy participants were enrolled and randomized (stratified by sex and needle experience) to inject etrolizumab into the abdomen or anterior thigh. All participants completed the study; however, 1 volunteer (needle-experienced, thigh injection) did not receive a full dose of etrolizumab because of a usage error (will be discussed further). The enrolled population was broadly representative of the IBD population. The median age of enrolled participants was 36 years, the mean BMI was 26.1 kg/m², and the majority of the participants were white (60%) and not Hispanic or Latino (83%). Approximately half (47%) of the participants were male.

Pain and Tolerability. Half of the participants did not report pain at any timepoint following injection (*see* Figure 15). For those participants who reported pain, all but one reported "very mild" or "mild" pain, the majority of which subsided within 60 minutes after drug administration. A single volunteer reported greater than mild pain (VDS-7 = 5) immediately following the injection, which subsided to mild pain at 5 minutes following injection. Similar data were reported when using the VAS (data not shown). Reported pain differed between injection sites. Injection into the thigh led to a greater proportion of participants reporting pain compared with participants injecting into the abdomen (60% vs 40%, respectively) (*see* Figure 16). The single volunteer reporting greater than mild pain was assigned to the thigh administration group. Participants injecting into the thigh also reported a longer duration of pain than those injecting into the abdomen; all pain experienced following abdominal injection subsided within 5 minutes, and the majority of pain following thigh injection subsided within 60 minutes. Needle-experience training did not appear to impact reported pain following etrolizumab injection. Using the intensive, LISSA-based monitoring scheme described

above, 40% of participants (12/30) experienced an ISR during the study, all of which occurred within 1 hour following etrolizumab injection (Table 1).

Table 1. Summary of local injection site symptom assessments over time by injection site and needle-experience groups

ISR	Timepoint, min	Reaction size, mm	Abdomen			Thigh			Overall (n = 30)
			Needle-naïve (n = 6)	Needle-experienced (n = 9)	Total (n = 15)	Needle-naïve (n = 6)	Needle-experienced (n = 9)	Total (n = 15)	
Hive formation	60	18		1 (11.1)	1 (6.7)				1 (3.3)
Redness	5	18	1 (16.7)		1 (6.7)				1 (3.3)
		21		1 (11.1)	1 (6.7)	2 (33.3)		2 (13.3)	3 (10.0)
		24	1 (16.7)		1 (6.7)				1 (3.3)
	60	<18		1 (11.1)	1 (6.7)				1 (3.3)
		18		1 (11.1)	1 (6.7)				1 (3.3)
		24				2 (23.3)	1 (11.1)	3 (20.0)	3 (10.0)
		31				1 (16.7)		1 (6.7)	1 (3.3)

5 ISR injection site reaction

All reported ISRs were mild (grade 1) and transient; all resolved by study completion. The most frequent ISR was redness, which ranged from < 18 to 31 mm in diameter. Most ISRs resolved within 60 minutes following injection. One volunteer reported formation of hives (18 mm in diameter) at the abdominal injection site 60 minutes postdose; the hives resolved within 3 hours without treatment. Injection site did not appear to affect either the frequency or severity of ISRs.

Usage Errors. Twenty-seven of the 30 participants (90%) were able to successfully self-administer etrolizumab using the AI without significant usage errors regardless of needle experience training. No complaints about the AI were registered, and no pattern of usage errors was observed. A total of 3 usage errors were observed during the study, only 1 of which occurred during the injection. One volunteer began to remove the AI prematurely during the injection, resulting in a droplet of liquid remaining on the volunteer's skin. Of the 2 usage errors not occurring during injection, 1 volunteer was unsure when to remove the cap from the AI, and another incorrectly reported the simulated expiration date. These 2 usage errors were associated with misunderstanding of the labeling and IFU of the AI; neither of these errors impacted the dose of

etrolizumab being administered. Most participants noted that they found the AI easy to use. Participants reported that the audible and visual feedback mechanisms were helpful for determining when the injection had started and stopped, and to verify that a complete dose had been administered. However, some participants stated that it was difficult to view the visual spinning top while injecting into their abdomen. During IFU comprehension probes, some confusion was noted related to acceptable injection sites, medication warm-up time, and product storage.

Pharmacokinetics (Exploratory). On study day 8 (7 days following injection), the mean ($\pm$ SD) serum concentration of etrolizumab across all participants was 13.6 ($\pm$ 3.66) μ g/mL (median 13.8). Serum concentrations ranged from 5.8 μ g/mL to 20.0 μ g/mL, a roughly 31% between-participant variability. Neither injection site nor needle training appeared to affect serum etrolizumab concentration at day 8 based on the limited data set.

Safety. Twenty-nine participants (97%) received the full 105 mg dose of etrolizumab; 1 volunteer received approximately 90% of the 105 mg dose. Overall, single 105 mg SC doses of etrolizumab were safe and well-tolerated when self-administered using the AI. Around half of participants experienced a treatment-emergent adverse event (TEAE), most of which were related to the injection site. All TEAEs were mild (grade 1) in severity and had resolved by study completion. No significant changes were noted in clinical laboratory evaluations, vital sign measurements, body weight measurements, or 12-lead ECGs during this study. Interestingly, more TEAEs were reported by participants injecting into the abdomen compared with the thigh (19 vs 9 TEAEs, respectively). Fewer TEAEs were reported by needle-experienced participants than needle-naïve participants. A summary of TEAEs can be found in Table 2.

Table 2. Summary of treatment-emergent adverse events

	Abdomen			Thigh			Overall (n = 30)
	Needle-naïve (n = 6)	Needle-experienced (n = 9)	Total (n = 15)	Needle-naïve (n = 6)	Needle-experienced (n = 9)	Total (n = 15)	
Participants with any TEAE	3 (50.0)	5 (55.6)	8 (53.3)	5 (83.3)	3 (33.3)	8 (53.3)	16 (53.3)
Number of TEAEs	13	6	19	6	3	9	28
Suspected to be caused by study drug	2 (33.3)	2 (22.2)	4 (26.7)	3 (50.0)	1 (11.1)	4 (26.7)	8 (26.7)
Other causes	2 (33.3)	3 (33.3)	5 (33.3)	2 (33.3)	2 (22.2)	4 (26.7)	9 (30.0)
Severity							
Mild (grade 1)	3 (50.0)	5 (55.6)	8 (53.3)	5 (83.3)	3 (33.3)	8 (53.3)	16 (53.3)

	Abdomen			Thigh			Overall (n = 30)
	Needle-naïve (n = 6)	Needle-experienced (n = 9)	Total (n = 15)	Needle-naïve (n = 6)	Needle-experienced (n = 9)	Total (n = 15)	
Total	3 (50.0)	5 (55.6)	8 (53.3)	5 (83.3)	3 (33.3)	8 (53.3)	16 (53.3)

Values are n (%). TEAE treatment-emergent adverse event

Discussion

In healthy participants, a single, self-administered, SC dose of etrolizumab using the AI was well-tolerated and resulted in mild pain for the majority of participants. This study met its primary end point with only a single volunteer experiencing greater than mild pain following injection. Overall, the data presented here are consistent with data of AIs used in the treatment of other chronic diseases, including rheumatoid arthritis (RA) and chronic kidney disease. These studies suggest many patients prefer the convenience of an AI compared with a PFS. Patients commonly report that AIs are associated with less pain than PFS devices and perceive AIs as more portable and easier to use (Kivitz et al., *Clin Ther.* 2006;28(10):1619–29; Kivitz and Segurado, *Expert Rev Med Devices.* 2007;4(2):109–16; Borrás-Blasco et al., *Expert Opin Biol Ther.* 2010;10(3):301–7; Lim et al., *Clin Ther.* 2012;34(9):1948–53). A recent study in patients with UC reported similar findings, noting that around three-quarters of patients in the study preferred injection with an AI compared with a PFS (Vermeire et al., *Patient Prefer Adherence.* 2018;12:1193–202). In a recent multinational survey, 200 patients with RA and 100 nurses were asked to rate the relative importance of various components of AIs (Tischer and Mehl, *Patient Prefer Adherence.* 2018;12:1413–24). Both patients and nurses rated “easy to perform the self-injection with the pen (ie, autoinjector)” as the most important attribute. Other key attributes, as reported by both patients and nurses, included “injection needle is safely concealed in the injector body,” “audible feedback after completion of the injection,” and “visual feedback after completion of the injection”; all of these features are built into the etrolizumab AI. Similar results were reported in a European study of 220 patients with RA (Thakur et al., *Rheumatol Ther.* 2016;3(2):245–56). It is worth noting that the proportion of patients experiencing mild ISRs in this study is higher than was observed in the phase 2 EUCALYPTUS study, in which etrolizumab was administered by vial and syringe (Vermeire et al., *Lancet.* 2014;384(9940):309–18). It was believed that this likely reflects differences in the study design, as this study assessed tolerability intensively, using LISSA to actively monitor for ISRs at scheduled intervals and possibly resulting in overreporting of ISRs. This first-in-human study is relatively unique in that it combined tolerability assessments, actual-use human factor

assessments (such as usage errors), and an initial PK assessment into a single trial. This novel approach, in part, aimed to assess overall risk associated with AI in a way that would minimize the number of clinical studies necessary, hence reducing the overall time and cost of AI development. PK assessment was incorporated into the study

5 protocol with a single blood sample taken on study day 8, around the time of maximum serum concentration following a single SC dose. The intent of this exploratory PK assessment was to understand the intersubject exposure variability following etrolizumab SC delivery by AI. In addition, these preliminary PK data helped to evaluate potential differences in exposure following AI injection compared with the predicted exposure

10 using a model generated based on PK data from administration with a vial and syringe in patients with UC. Of note, the day 8 etrolizumab exposure observed in this analysis was approximately 75% higher than the predicted value (predicted day 8 median etrolizumab serum concentration ≈ 7.9 $\mu\text{g/mL}$ [90% CI 4.15–16.3], data not shown). The PK variability and unexpected higher day 8 exposure from this analysis informed the

15 decision to conduct a 2-part study comparing the pharmacokinetics of etrolizumab delivered by the AI and PFS-NSD in healthy volunteers (*see* Figure 17 and Example 3). Results from this study effectively eliminated the requirement for additional AI ease of use and/or clinical studies. In addition, these results influenced the design of a subsequent study to compare PK properties between administrations by the PFS-NSD

20 and AI, mitigating the risk of failing the comparability study and minimizing unnecessary exposure of healthy volunteers to biological treatment. As a result of the PK findings reported here, a pilot cohort was added to the originally proposed single-part device PK comparability study design. Results from this pilot cohort served to optimize the design of the pivotal cohort by informing the proper sample size, sample collection

25 duration, and body weight range. Information gained from the human factors component of this study resulted in small amendments to the IFU, implemented before the PK comparability study. Results from the device PK comparability are reported in Example 3.

Conclusion

30 Results from this study demonstrate that single SC injections of etrolizumab with an AI are well-tolerated in healthy volunteers, with tolerable levels of pain following injection. Most participants found the AI easy to use and experienced only minimal usage errors. The AI may be an appropriate delivery mechanism for certain patients with IBD who desire the safety and convenience of self-injection with an invisible needle.

The positive results from this first-in-human tolerability study, in combination with data gathered during the subsequent 2-part PK comparability study, comprise a complete development plan to support the use of AI in patients treated with etrolizumab.

**Example 3 – Comparable Pharmacokinetics, Safety, and Tolerability of
5 Etrolizumab Administered via Prefilled Syringe or Autoinjector in a Randomized
Trial in Healthy Volunteers**

Summary

Etrolizumab is a novel, dual-action anti- β 7 integrin antibody being studied in several phase 3 trials in patients with inflammatory bowel disease. An autoinjector (AI) device is being developed in parallel to complement the prefilled syringe (PFS) with
10 needle safety device (NSD) used for subcutaneous administration in these trials. This Example demonstrates the comparability of pharmacokinetics (PK), tolerability, and safety of both devices.

This randomized, open-label, 2-part study in healthy participants evaluated the comparability of etrolizumab exposure between the AI and PFS-NSD. Part 1 (pilot) involved a small number of participants, and initial results were used to finalize the study design of the larger part 2 (pivotal). In both parts, participants were randomly assigned to receive a single dose of subcutaneous (SC) etrolizumab 105 mg via AI or PFS-NSD. Randomization was stratified by body weight. The primary PK outcomes were $C_{\max}$,
15 AUC_{last} , and $AUC_{0-\text{inf}}$.

One hundred and eighty healthy participants (part 1: $n = 30$, part 2: $n = 150$) received a single SC dose of etrolizumab via AI or PFS-NSD. Primary PK results from part 1 supported modification of the part 2 study design. Results from part 2 demonstrated that etrolizumab exposure was equivalent between devices, with geometric
25 mean ratios (GMRs) between AI and PFS-NSD of 102% for $C_{\max}$, 98.0% for AUC_{last} , and 97.6% for $AUC_{0-\text{inf}}$. Median $T_{\max}$ and mean terminal $t_{1/2}$ were also similar between devices. The GMRs and 90% confidence intervals of all primary PK parameters were fully contained within the predefined equivalence limits (80% to 125%).

This PK study demonstrated that single SC injections of etrolizumab 105 mg using an AI or PFS-NSD result in equivalent etrolizumab exposure and similar safety
30 and tolerability in healthy participants. Taken together, these results support the use of an AI for etrolizumab administration.

Introduction

The PK comparability study (NCT02996019) presented in this Example aimed to demonstrate the comparability of etrolizumab exposure following SC administration using the AI and PFS-NSD, and to evaluate the safety and tolerability of etrolizumab following SC injection using the 2 devices. Part 1 of the study was an exploratory pilot cohort used to evaluate the geometric mean ratio (GMR) and variability of PK parameters for etrolizumab administration with the AI versus PFS-NSD. Those results informed the study design including sample size and study duration for part 2 (the pivotal cohort). In part 2, the study aimed to demonstrate exposure comparability between a single dose of etrolizumab administered via AI or PFS-NSD. The PK comparability study presented in this Example leveraged the exploratory PK results from the tolerability study presented in Example 2 to refine the study design and final protocol.

Methods

Study Design and Procedures. This study was a randomized, multicenter, open-label, parallel-group study conducted in healthy participants at three clinical sites within the United States (see Figure 18). This 2-part study consisted of a pilot cohort (part 1) and a pivotal cohort (part 2) with a sample size sufficient for 80% power to detect the exposure difference (if any) between the 2 device groups. In both parts, healthy participants were randomly assigned 1:1 to receive a single dose of etrolizumab 105 mg SC via either AI (test device) or PFS-NSD (reference device). Etrolizumab was administered by a health care professional (HCP) into the participant's abdomen. Randomization in both cohorts was stratified by body weight (≤ 79.9 vs ≥ 80 kg).

Participants. Eligible healthy participants included men and women between 18 and 55 years of age with a body mass index (BMI) between 18.0 and 30.0 kg/m². Based on results from the pilot cohort, it was also required that participants in the pivotal cohort have a body weight within the range of 60 to 100 kg (inclusive) at the time of study entry. Participants must have been in good health (no clinically significant findings from medical history, physical examination, 12-lead electrocardiogram, or vital signs). Participants with any prior exposure to immunosuppressants or anti-integrin therapies (including etrolizumab) were excluded.

Assessments and Outcome Measures. Blood samples for determination of etrolizumab serum concentrations in both part 1 and part 2 were collected predose and 6 hours postdose on day 1, then on days 2, 4, 6, 8, 11, 15, 29, 43, 57, and 71 (study completion). The geometric mean ratio (GMR) and variability of the maximum

etrolizumab concentration ($C_{\max}$), area under the concentration-time curve from the time of drug administration to the last measurable concentration (AUC_{last}), AUC extrapolated to infinity ($AUC_{0-\text{inf}}$), as well as the ratio of AUC_{last} to $AUC_{0-\text{inf}}$ (AUCR) of etrolizumab, were measured in part 1. For part 2, $C_{\max}$, AUC_{last} , and $AUC_{0-\text{inf}}$ were measured as
5 primary endpoints. Secondary PK parameters included the time to maximum concentration of etrolizumab ($t_{\max}$), the terminal elimination half-life ($t_{1/2}$), and AUCR. Blood samples for determination of antidrug antibodies (ADAs) in parts 1 and 2 were collected before dosing on day 1, and on days 29, 57, and 71. Data from all ADA-positive participants were included in the final PK statistical analysis unless the
10 participant met the PK analysis predefined exclusion criteria. Safety and tolerability assessments included the incidence, nature, and severity of adverse events, graded according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03. The incidence of injection-site reactions, changes in vital signs, physical examination findings, clinical laboratory results, and the incidence of ADAs
15 were also assessed.

Bioanalytical methods. Etrolizumab concentrations were measured using a validated immunoassay (ICON). This Gyrolab fluorescence immunoassay utilized a minimum required dilution (MRD) of 1/100, with a minimum quantifiable concentration of 80 ng/mL etrolizumab in UC, CD, and healthy volunteer sera. Anti-etrolizumab
20 antibodies in serum were detected using a validated assay. This colorimetric ELISA used a 1/20 MRD and a monoclonal anti-etrolizumab control antibody. Relative sensitivity of the method was determined to be 12.0 ng/mL in healthy volunteer serum. Drug tolerance of the assay was established: 28 ng/mL of the positive control ADA could be detected in the presence of 50 µg/mL etrolizumab.

Sample Size Determination. Enrollment of up to 30 healthy participants in part 1 was planned to ensure at least 12 participants in each arm (24 total) had evaluable PK profiles to enable estimation of the GMR and coefficient of variation (CV%) for PK parameters ($C_{\max}$, AUC_{last} , and $AUC_{0-\text{inf}}$). Part 2 planned for enrollment of 146
25 participants. Assuming a dropout rate or non-evaluable PK profiles from approximately 10% of participants through day 71, a total of approximately 131 healthy participants with evaluable full PK profiles were expected to provide at least 80% power to
30 demonstrate exposure comparability for $C_{\max}$, AUC_{last} , and $AUC_{0-\text{inf}}$ based on the GMR and PK variability outcomes from the part 1 pilot cohort.

Pharmacokinetic analyses. PK parameters were determined from the serum etrolizumab concentrations using noncompartmental methods (NCA) and were performed using Phoenix WinNolin (Centara USA, Inc., Version 6.4).

Statistical Analyses. The analysis population consisted of all participants who received an SC injection of etrolizumab and who had an evaluable PK profile, which was defined as having sufficient samples available to accurately determine key PK parameters. In particular, participants with early termination on or before day 15 were considered not having an evaluable PK profile. Participants with no sample available to determine the concentration of day 71 were excluded from statistical analysis of AUC_{last} ; participants with < 3 available samples among days 28, 43, 51, and 71 were excluded from statistical analysis of AUC_{0-inf} . Participants with predose concentration $> 5\%$ of C_{max} may be excluded from statistical analysis of all PK parameters at the discretion of the study and sponsor clinical pharmacokineticist/biostatistician. Descriptive, exploratory analysis of PK parameters was carried out with data from part 1 (pilot), with a focus on evaluating GMRs, CV%, and distribution of AUCR to inform the part 2 (pivotal) sample size and final study design. Only data from the pivotal cohort (part 2) were included in comparability statistical analysis. In part 2, an analysis of variance, including treatment as the fixed effect, was performed to assess comparability of C_{max} , AUC_{last} , and AUC_{0-inf} between the AI and PFS-NSD groups. Data for C_{max} , AUC_{last} , and AUC_{0-inf} were natural log (ln)-transformed before analysis, and the 90% confidence intervals (CIs) of the GMRs for the AI group relative to those from the PFS-NSD group were calculated by taking the antilog of the corresponding 90% CIs for the differences between the means (log scale). Exposure between the AI and PFS-NSD groups met PK comparability criteria if the 90% CIs of the GMRs for C_{max} , AUC_{last} , and AUC_{0-inf} were all within 80% to 125%.

Results

Pharmacokinetics—Pilot Study (Part 1). All 30 participants enrolled and randomized in part 1 received a single 105 mg SC dose of etrolizumab via AI ($n = 15$) or PFS-NSD ($n = 15$). Twenty-seven participants completed the study; 2 participants (1 in each arm) discontinued earlier because of loss of follow-up and 1 discontinued because of a serious AE (seizure) 32 days following administration of etrolizumab (PFS-NSD arm); this AE was not considered related to etrolizumab treatment. Participant characteristics are shown in Table 3.

Table 3. Participant characteristics

	Pilot cohort (part 1)		Pivotal cohort (part 2)	
	AI n = 15	PFS-NSD n = 15	AI n = 74	PFS-NSD n = 76
Mean age (min, max), years	42 (28, 54)	34 (20, 53)	35 (21, 55)	37 (19, 55)
Mean weight (min, max), kg	79.7 (65.1, 108.8)	74.3 (50.8, 92.1)	76.2 (61.0, 99.4)	76.3 (55.90, 97.5)
Mean height (min, max), cm	172.0 (154.3, 190.8)	173.5 (154.6, 194.9)	170.3 (151.2, 193.4)	170.7 (151.5, 189.0)
Mean BMI (min, max), kg/m ²	26.8 (23.2, 29.9)	24.5 (20.6, 29.2)	26.3 (20.4, 30.0)	26.2 (19.2, 29.9)
Sex, n (%)				
Male	9 (60.0)	10 (66.7)	40 (54.1)	40 (52.6)
Female	6 (40.0)	5 (33.3)	34 (45.9)	36 (47.4)
Race, n (%)				
Asian	0	0	2 (2.7)	1 (1.3)
Black/African American	5 (33.3)	4 (26.7)	31 (41.9)	23 (30.3)
White	10 (66.7)	11 (73.3)	40 (54.1)	51 (67.1)
Unknown	0	0	1 (1.4)	1 (1.3)
Ethnicity, n (%)				
Hispanic or Latino	6 (40.0)	3 (20.0)	14 (18.9)	14 (18.4)
Not Hispanic or Latino	9 (60.0)	12 (80.0)	60 (81.1)	62 (81.6)
JCV antibody-positive, n (%)	7 (46.7)	8 (53.3)	38 (51.4)	46 (60.5)

AI autoinjector. BMI body mass index. JCV John Cunningham virus. PFS-NSD prefilled syringe with needle safety device.

In the overall cohort, most participants were male (63.3%) and white (70.0%). The body weight and age of participants were not well-balanced between treatment arms; the mean body weight was 79.7 kg in the AI arm and 74.3 kg in the PFS-NSD arm, and the mean age was 42 years in the AI arm and 34 years in the PFS-NSD arm. Since body weight appears to impact etrolizumab exposure, especially AUC_{0-inf} (see Figure 19), the imbalance in body weight between treatment arms could potentially bias assessment of GMR values of primary PK parameters and PK variability. Therefore, participants in the pilot cohort with a body weight lower than 60 kg (n=4) or higher than 100 kg (n=1) were excluded from the exploratory analysis aiming to evaluate GMR and CV% of PK parameters which were used to guide the determination of final sample size for pivotal study (part 2). The PK evaluable population meeting the body weight restriction of 60 to 100 kg in part 1 included 14 participants in the AI arm and 11 participants in the PFS-NSD arm. As expected, when body weight range between the two arms was balanced via restricting body weight to a 60-100kg range, AI versus PFS-NSD group primary PK parameter GMR values were 0.95, 1.02, and 1.02 for C_{max}, AUC_{last}, and AUC_{0-inf}, respectively (Table 4).

Table 4. Summary of PK parameters in cohort 1 participants meeting the body weight restriction of 60 to 100 kg

Parameter	AI (test)		PFS-NSD (reference)		GMR (%)	Pooled CV%
	n ^a	Geometric mean ^b	n ^a	Geometric mean ^b		
C _{max} (µg/mL)	14	10.6	11	11.1	0.955	30.1
AUC _{last} (day·µg/mL)	12	273.3	9	267.9	1.020	34.1
AUC _{0-inf} (day·µg/mL)	14	284.6	10	278	1.024	34.0

AI autoinjector. ANOVA analysis of variance. AUC_{0-inf} AUC extrapolated to infinity. AUC_{last} area under the concentration-time curve from the time of drug administration to the last measurable concentration (last is Day 71 for all available data). C_{max} maximum concentration. GMR geometric mean ratio. PFS-NSD prefilled syringe with needle safety device.

5 ^aNumber of observations in each treatment eligible for analysis.

^bGeometric means are based on the least-squares means for C_{max} and AUC parameters from ANOVA, calculated by transforming the natural log means back to the linear scale.

Hence, the sample size for pivotal cohort was calculated based on these GMR and CV% values, and a body weight restriction was added for the part 2 study as an inclusion criterion. Furthermore, all participants had AUCR values > 80% (data not shown), which meets the requirement for bioequivalence study stated in the US Food and Drug Administration (FDA) guideline (US Department of Health and Human Services. Guidance for industry: statistical approaches to establishing bioequivalence. Accessed March 4, 2020), and hence resulted in a modification of the last day of the pivotal study from originally planned day 85 to day 71.

Pharmacokinetics—Pivotal Study (Part 2). In the pivotal cohort, 150 (100%) of the enrolled and randomized participants received a single SC 105 mg dose of etrolizumab via AI (n = 74) or PFS-NSD (n = 76). Eight participants discontinued the study; 5 because of loss of follow-up (3 in the AI arm and 2 in the PFS-NSD arm), 1 because of a protocol violation in not meeting body weight criteria (PFS-NSD), and 2 (1 in each arm) because of participant withdrawal. In the overall part 2 (including both arms), 53.5% of participants were male, 60.7% were white, and 36.0% were African American. Treatment arms were well-balanced for body weight and age; mean body weight was 76.2 kg in the AI arm and 76.3 kg in the PFS-NSD arm, and mean age was 35 years in the AI arm and 37 years in the PFS-NSD arm (Table 3).

Etrolizumab serum concentrations over time for the AI and PFS-NSD groups are shown in Figure 20; PK parameters are summarized in Table 5. GMRs (90% CIs) between the AI and PFS-NSD groups were 102% (94.2–111%) for C_{max}, 98.0% (89.3–107%) for AUC_{last}, and 97.6% (88.6–107%) for AUC_{0-inf} (Table 6).

30 Table 5. Summary of PK parameters for etrolizumab in the pilot and pivotal studies

Parameter	Pilot cohort (part 1) ^a				Pivotal cohort (part 2)			
	n	AI (n = 14)	n	PFS-NSD (n = 11)	n	AI (n = 76)	n	PFS-NSD (n = 74)
C _{max} (µg/mL)	14	10.6 (28.3%)	10	11.0 (35.3%)	73	12.1 (32.0%)	73	12.2 (28.3%)
t _{max} ^b (day)	14	5.00 (2.95, 9.94)	10	3.98 (2.90, 6.97)	73	5.04 (2.98, 14.0)	73	6.97 (3.00, 14.0)
AUC _{last} (day·µg/mL)	13	272 (39.8%)	9	268 (27.0%)	69	319 (35.3%)	72	325 (33.1%)
AUC _{0-inf} (day·µg/mL)	13	284 (41.6%)	10	278 (27.0%)	69	329 (36.0%)	72	337 (35.2%)
t _{1/2} ^c (day)	13	12.2 (4.90)	10	11.4 (4.04)	69	11.8 (3.85)	72	12.2 (4.39)
CL/F (L/day)	13	0.369 (41.6%)	10	0.378 (27.0%)	69	0.319 (36.0%)	72	0.311 (35.2%)
AUCR ^d	13	0.957 (3.5%)	9	0.975 (2.6%)	67	0.966 (3.4%)	72	0.964 (3.7%)
≤80%		0		0		0		1 (1%)
>80%		13 (100%)		9 (100%)		67 (100%)		71 (99%)

AI autoinjector. AUC_{0-inf} AUC extrapolated to infinity. AUC_{last} area under the concentration-time curve from the time of drug administration to the last measurable concentration. AUCR ratio of AUC_{last} to AUC_{0-inf}. C_{max} maximum concentration. PFS-NSD prefilled syringe with needle safety device. PK pharmacokinetic. t_{1/2} terminal elimination half-life. t_{max} time to maximum concentration.

5 Geometric mean (geometric CV%) data are presented unless otherwise indicated.

^aIncludes only participants who met body weight restrictions (60 to 100 kg) in the pilot cohort.

^bMedian (min, max) presented for t_{max}. ^cArithmetic mean (SD) presented for t_{1/2}. ^dn (%) presented.

Table 6. Outcomes of ANOVA to assess comparability of PK parameters for AI versus PFS-NSD (pivotal cohort)

Parameter	AI (test)		PFS-NSD (reference)		GMR ^c (%)	GMR 90% CI ^d (%)
	n ^a	Geometric mean ^b	n ^a	Geometric mean ^b		
C _{max} (µg/mL)	73	12.5	73	12.2	102	94.2–111
AUC _{last} (day·µg/mL)	69	319	72	325	98.0	89.3–107
AUC _{0-inf} (day·µg/mL)	69	329	72	337	97.6	88.6–107

AI autoinjector. ANOVA analysis of variance. AUC_{0-inf} AUC extrapolated to infinity. AUC_{last} area under the concentration-time curve from the time of drug administration to the last measurable concentration. C_{max} maximum concentration. GMR geometric mean ratio. PFS-NSD prefilled syringe with needle safety device.

^aNumber of observations in each treatment eligible for analysis.

15 ^bGeometric means are based on the least-squares means for C_{max} and AUC parameters from ANOVA, calculated by transforming the natural log means back to the linear scale.

^cGeometric mean ratio for test/reference ratio of parameter means for natural log-transformed parameter (expressed as a percent). Natural log-transformed ratios transformed back to the linear scale.

^d90% confidence interval for ratio of parameter means of natural log-transformed parameter (expressed as a percent).

20 Natural log-transformed confidence limits transformed back to the linear scale.

The 90% CIs of the GMRs for each of these primary PK parameters were within the predefined equivalence limits of 80% to 125%, which meets the predefined comparability criteria and supports equivalent exposure of etrolizumab between 2 device groups. The results also demonstrated similar median time to maximum observed concentration ($t_{\max}$, 5.04 vs 6.97 days) and mean terminal elimination half-life ($t_{1/2}$, 11.8 vs 12.2 days) between AI and PFS-NSD groups.

Safety. The overall incidence of treatment-emergent ADAs among post-baseline evaluable participants was 20.7% (6/29) in the pilot cohort and 29.7% (44/148) in the pivotal cohort, and was similar when comparing the AI and PFS-NSD groups in both the pilot cohort (3/15 [20%] vs 3/14 [21.4%]) and the pivotal cohort (20/73 [27.4%] vs 24/75 [32.0%]). PK profiles of ADA+ subjects appear to be similar to those ADA negative subjects (*see* Figure 21). Treatment-emergent adverse events (TEAEs) were experienced by 53% of participants in the pilot cohort and 35% in the pivotal cohort, and most were mild in severity (Table 7).

Table 7. Treatment-Emergent Adverse Events (TEAE)

	Pilot cohort (part 1)			Pivotal cohort (part 2)			Overall (parts 1 and 2) n = 180
	AI n = 15	PFS- NSD n = 15	Total n = 30	AI n = 74	PFS- NSD n = 76	Total n = 150	
Any TEAE	9 (60.0)	7 (46.7)	16 (53.3)	22 (29.7)	27 (35.5)	49 (32.7)	65 (36.1)
Number of TEAEs	22	7	29	32	45	77	106
Any SAE	0	1 (6.7)	1 (3.3)	0	0	0	1 (0.6)
Any ISR-related TEAE	5 (33.3)	4 (26.7)	9 (30.0)	1 (1.4)	0	1 (0.7)	10 (5.6)
Suspected to be caused by							
Study drug	6 (40.0)	3 (20.0)	9 (30.0)	7 (9.5)	10 (13.2)	17 (11.3)	26 (14.4)
Other causes	7 (46.7)	2 (26.7)	11 (36.7)	18 (24.3)	21 (27.6)	39 (26.0)	50 (27.8)
Concurrent Illness	4 (26.7)	0	4 (13.3)	7 (9.5)	14 (18.4)	21 (14.0)	25 (13.9)
Concomitant medication	0	0	0	2 (2.7)	1 (1.3)	3 (2.0)	3 (1.7)
Procedures	3 (20.0)	1 (6.7)	4 (13.3)	0	1 (1.3)	1 (0.7)	5 (2.8)
Other	2 (13.3)	3 (20.0)	5 (16.7)	10 (13.5)	6 (7.9)	16 (10.7)	21 (11.7)
Missing	0	0	0	1 (1.4)	0	1 (0.7)	1 (0.6)
AE by most severe NCI CTCAE grade							
Grade 1	8 (53.3)	4 (26.7)	12 (40.0)	19 (25.7)	27 (35.5)	46 (30.7)	58 (32.2)
Grade 2	1 (6.7)	2 (13.3)	3 (10.0)	3 (4.1)	0	3 (2.0)	6 (3.3)
Grade 3	0	1 (6.7)	1 (3.3)	0	0	0	1 (0.6)
Total	9 (60.0)	7 (46.7)	16 (53.3)	22 (29.7)	27 (35.5)	49 (32.7)	65 (36.1)

AE adverse event; *AI* autoinjector, *ISR* injection site reaction, *NCI CTCAE* National Cancer Institute Common Terminology Criteria for Adverse Events, *PFS-NSD* prefilled syringe with needle safety device, *SAE* serious adverse event, *TEAE* treatment-emergent adverse event, Data are reported as n (%) unless otherwise specified.

One participant in the PFS-NSD arm of the pilot cohort had a serious AE (grade 3 seizure) approximately 32 days following injection that led to study discontinuation and was not considered related to treatment with etrolizumab. Nine participants (5 with AI and 4 with PFS-NSD) in the pilot cohort and 1 participant (AI arm) in the pivotal cohort had injection site reactions that were reported as TEAEs. Treatment-related TEAEs in the pilot cohort were experienced by 6 (40.0%) participants in the AI arm and 3 (20.0%) in the PFS-NSD arm, and in the pivotal cohort by 7 (9.5%) in the AI arm and 10 (13.2%) in the PFS-NSD arm.

Discussion

The pivotal cohort in this study confirmed comparable etrolizumab exposure between AI and PFS-NSD groups following a single dose of SC etrolizumab in healthy participants. The GMRs observed with each of the primary PK parameters were between 98% and 102%, with 90% CIs within the prespecified equivalence limits. These GMRs for all primary exposure parameters are impressive, given the GMRs obtained when comparing other AIs and PFS-NSDs in healthy participants. For example, a recent study comparing AI and PFS devices for the SC administration of an adalimumab biosimilar (SB5) demonstrated GMRs of 102%, 107%, and 110% for C_{max} , AUC_{last} , and AUC_{inf} , respectively, with 90% CIs within equivalence limits of 80% to 125%. In a similar study of the adalimumab biosimilar BI 695501 they were 100% (90% CI 82.1–122.3%) for AUC_{0-inf} and 110% (90% CI 96.8–125.4%) for C_{max} , the latter of which was above the equivalence upper 90% CI equivalence limit of 125% (Voltaire®-AI study) (Ramael et al., *Rheumatol Ther.* 2018;5(2):403–21; Shin et al., *Drug Des Devel Ther.* 2018;12:3799–805). The pilot cohort was valuable to inform the final design of the pivotal cohort. The pilot cohort mainly evaluated GMRs and the variability of PK parameters, which are the key assumptions used in the sample size estimation. To minimize the risk of underpowering the pivotal PK comparability study, a small pilot cohort was added to the original study protocol with the intent of gaining certainty around GMR values of the primary PK parameters and PK variability following administration of etrolizumab by AI or PFS-NSD. The GMR and PK variability values obtained from the pilot cohort (see Table 4) provided added confidence in estimating the sample size for the pivotal cohort. Although body weight was stratified at

randomization, the final body weight distribution range was still imbalanced in the pilot cohort, which may have biased the final GMR outcome. To minimize such bias, only data from participants with a body weight within the range of 60 to 100 kg (a common body weight range for both arms within the pilot cohort) were used for the estimation of GMR and PK variabilities. This body weight restriction of 60 to 100 kg was also implemented in the pivotal cohort as an inclusion criterion. The pilot cohort also evaluated a study duration of 10 weeks (70 days), 2 weeks shorter than the original study planned study duration as suggested by the FDA. As expected, all evaluable participants in the pilot cohort had AUCR values > 80%, the value required by the FDA for PK comparability studies. This result suggested that the 10-week study duration is long enough to capture more than 80% of AUC_{inf} and hence the duration of the pivotal study could be shortened from 12 weeks to 10 weeks without the risk of missing the requirement of < 20% extrapolated AUC for the calculated AUC_{inf}. Immunogenicity was relatively high in this study compared with other studies using etrolizumab. The rate of ADAs was > 20% in both the pilot and pivotal cohorts, whereas 5% (2/38) of patients with UC who received single or multiple etrolizumab doses in a phase 1 trial (Rutgeerts et al., *Gut*. 2013;62(8):1122–30) and 5% (4/81) of patients with UC who received multiple etrolizumab doses in a randomized phase 2 trial (Vermeire et al., *Lancet*. 2014;384(9940):309–18) had detectable ADAs following etrolizumab treatment. Immunogenicity incidence rate in the pilot and pivotal cohorts was equivalent in both PFS-NSD and AI groups. The higher ADA rate in the current study may have been the result of differences in study design: the current study had a single SC dose regimen and a study population of healthy participants not taking immunosuppressive drugs. In comparison, patients in prior trials of etrolizumab had insufficiently controlled, moderate-to-severe UC and were commonly being treated with immunosuppressive agents. Although the incidence of ADAs after a single SC injection of etrolizumab was relatively high (>20%) in this study, the impact of ADA positivity on PK appears to be minimal, given that the PK profiles of ADA-positive participants largely overlapped with those observed in ADA-negative participants (Figure 21). Moreover, the variability (CV%) of AUC_{0-inf} or AUC_{last} values ranged from 33% to 36% in the pivotal part 2 study, which was in line with those observed for other monoclonal antibodies (Ramael et al., *Rheumatol Ther*. 2018;5(2):403–21; Anumolu et al., *Clin Pharmacol Drug Dev*. 2018;7(8):829–36). Such a small exposure variability further confirms that the total exposure (AUC_{0-inf}) between ADA-positive and ADA-negative participants was very

similar. Data from all evaluable participants, regardless of their ADA status, were included in the comparability statistical assessment, and the final outcome showed a high degree of exposure similarity between groups. The bioequivalence testing of the AI and PFS-NSD groups and sample size determination in the pivotal study design following a single SC dose of etrolizumab were based on data from a subset of participants within a body weight range of 60 to 100 kg. Although our finding of comparable etrolizumab exposure between the AI and PFS-NSD was based on data from participants within a defined body weight range, it can be applied to individuals outside this body weight range, given that the current study identified no exposure difference solely due to the drug delivery device. Furthermore, a similar study for another therapeutic antibody (that did not restrict by body weight) suggests that this may be a fair assumption (Ramael et al., *Rheumatol Ther.* 2018;5(2):403–21). In that study, a similar relationship between drug exposure and body weight was observed, but when comparing AI and PFS-NSD devices for adalimumab SC administration, bioequivalence was still achieved regardless of body weight. Clearance of etrolizumab is known to be significantly impacted by body weight (Tang et al., *Aliment Pharmacol Ther.* 2018;47(11):1440–52), a finding confirmed in the pilot cohort of this study. In these healthy participants, a single SC dose of etrolizumab was generally safe and well-tolerated when administered using either an AI or PFS-NSD. Treatment-related TEAEs were comparable with the AI versus the PFS-NSD in the pivotal cohort (9.5% vs 13.2%). The majority of TEAEs were mild or moderate in severity, and mostly resolved by the end of the study. One serious AE (seizure) led to study discontinuation in the PFS-NSD arm of the pilot cohort; however, this was not considered to be related to treatment with etrolizumab.

Conclusion

Results from this PK comparability study demonstrate that etrolizumab exposure is similar when administered SC via either AI or PFS-NSD, with 90% CI for exposure parameters (AUC_{last} , C_{max} , AUC_{inf}) contained within bioequivalence limits (80–125%) between devices. In addition, single SC doses of etrolizumab administered via AI were generally well-tolerated in healthy participants and were not associated with increased adverse events compared with PFS-NSD injection. This study also highlights the value of a pilot cohort in facilitating the design of a pivotal PK comparability study, as data from the pilot cohort increased confidence in the study design and minimized assumption bias.

Example 4

This Example provides details relating to PFS-NSD injection forces.

There are multiple force definitions during the injection experience for the user. The user actions are: 1) remove the needle cover, and 2) press the plunger until the needle safety is activated. Figure 22 shows the different force definitions. Table 8 shows the forces and their limits. Table 9 shows that the injection forces are within limits for both 1mL and 2.25mL configurations.

Table 8

Force	Limits
Average Injection Force (aka Glide Force)	≤26 Newton (N)
Break Loose Force	≤33N
Maximum Injection Force	≤33N

10

Table 9

Attribute	Real time aging (2-8 C) Time is shown in months below							High Temperature (25 C)	
	T=initial	T=6	T=12, 24					T=3	T=6
Glide Force	Within limits							Within limits	
Break Loose Force	Within limits							Within limits	
Max Injection Force	Within limits							Within limits	

There is a spring mechanism that pushes the liquid out for the autoinjector. Therefore, injection time is relevant attribute. In certain embodiments the injection time is between about 0.4 seconds and about 9 seconds. In certain embodiment, the injection time is about 5 seconds.

The autoinjector used for the presently disclosed formulation provides advantageous injection times and extended stability.

Embodiments of the presently disclosed subject matter

From the foregoing description, it will be apparent that variations and modifications may be made to the presently disclosed subject matter to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or sub-combination) of listed elements. The recitation of an embodiment herein includes that

embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and
5 publication was specifically and individually indicated to be incorporated by reference.

WHAT IS CLAIMED IS:

1. A formulation comprising a monoclonal anti-integrin beta7 antibody, wherein in the concentration of the anti-integrin beta7 antibody is at least about 100 mg/ml, the viscosity of the formulation is less than about 20 centipoise (cP) at 25°C, and wherein the formulation has extended stability.
2. The formulation of claim 1, wherein the anti-integrin beta7 antibody is a humanized antibody.
3. The formulation of claim 1, wherein the viscosity of the formulation is about 7 cP at 25°C.
4. The formulation of any one of claims 1-3, wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:
 - (i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;
 - (ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO:2;
 - (iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3;
 - (iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4;
 - (v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and
 - (vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or SEQ ID NO:7.
5. The formulation of any one of claims 1-4, wherein the anti-integrin beta7 antibody comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 8, and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9.
6. The formulation of any one of claims 1-5, wherein the anti-integrin beta7 antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 11.

7. The formulation of any one of claims 1-5, wherein the anti-integrin beta7 antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 12.
8. The formulation of any one of claims 1-7, wherein the anti-integrin beta7 antibody is etrolizumab.
9. The formulation of any one of claims 1-8, wherein the concentration of the anti-integrin beta7 antibody in the formulation is between about 100 mg/ml and about 220 mg/ml.
10. The formulation of any one of claims 1-9, wherein the concentration of the anti-integrin beta7 antibody in the formulation is 150 mg/mL or about 150 mg/ml.
11. The formulation of any one of claims 1-11, wherein the pH of the formulation is greater than 5.5.
12. The formulation of claim 12, wherein the pH of the formulation is between 5.65 and 6.1.
13. The formulation of any one of claims 1-12, wherein the pH of the formulation is 5.8, between 5.7 and 5.9 or between 5.75 and 5.85.
14. The formulation of any one of claims 1-13, further comprising a surfactant, wherein the concentration of the surfactant in the formulation is between 0.03% w/v and 0.06% w/v.
15. The formulation of claim 14, wherein the concentration of the surfactant in the formulation is 0.04% w/v or about 0.04% w/v.
16. The formulation of claim 14 or 15, wherein the surfactant is polysorbate 20.
17. The formulation of any one of claims 1-16, further comprising arginine succinate.
18. The formulation of claim 17, wherein the concentration of arginine succinate in the formulation is between about 100 mM and about 300 mM.

19. The formulation of claim 17 or 18, wherein the concentration of the arginine succinate in the formulation is between about 150 mM and about 300 mM.
20. The formulation of any one of claims 17-19, wherein the concentration of the arginine succinate in the formulation is between about 150 mM and about 250 mM.
21. The formulation of any one of claims 17-20, wherein the concentration of arginine succinate in the formulation is 200 mM or about 200 mM.
22. The formulation of any one of claims 1-21, further comprising histidine.
23. The formulation of claim 22, wherein the concentration of histidine in the formulation is between about 5 mM and about 40 mM.
24. The formulation of claim 22 or 23, wherein the concentration of histidine in the formulation is 20 mM or about 20 mM.
25. The formulation of any one of claims 1-24, wherein the anti-integrin beta7 antibody is stable for at least about seven years at -20°C.
26. The formulation of any one of claims 1-25, wherein the anti-integrin beta7 antibody is stable for at least about 18 months at 5 °C.
27. The formulation of any one of claims 1-26, wherein the anti-integrin beta7 antibody is stable for at least about two years at 5 °C.
28. The formulation of any one of claims 1-27, wherein the anti-integrin beta7 antibody is stable for at least about 1 day at room temperature.
29. The formulation of any one of claims 1-28, wherein the anti-integrin beta7 antibody is stable for up to about 1 month at room temperature.
30. A formulation comprising an anti-integrin beta7 antibody, in 20 mM histidine buffer or about 20 mM histidine buffer, pH 5.8 or pH between 5.7 and 5.9 or pH between 5.75 and 5.85, 0.04% polysorbate 20 or about 0.04% polysorbate 20, and 200 mM arginine succinate or about 200 mM arginine succinate, wherein the concentration of the anti-integrin beta7 antibody is 150 mg/mL or about 150 mg/ml, and wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-

L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

- (i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;
- (ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO:2;
- (iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3;
- (iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4;
- (v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and
- (vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or

SEQ ID NO:7.

31. The formulation of claim 30, wherein the anti-integrin beta7 antibody comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 8, and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9.

32. The formulation of claim 30 or 31, wherein the anti-integrin beta7 antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 11.

33. The formulation of claim 30 or 32, wherein the anti-integrin beta7 antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 12.

34. The formulation of any one of claims 30, 32, and 33, wherein the anti-integrin beta7 antibody is etrolizumab.

35. A formulation comprising an anti-integrin beta7 antibody, in 20 mM histidine buffer or about 20 mM histidine buffer, pH 5.8 or pH between 5.7 and 5.9 or pH between 5.75 and 5.85, 0.04% polysorbate 20 or about 0.04% polysorbate 20, and 200 mM arginine succinate or about 200 mM arginine succinate, and wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

- (i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;
- (ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO:2;
- (iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3;

- (iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4;
- (v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and
- (vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or SEQ ID NO:7.

36. The formulation of claim 35, wherein the anti-integrin beta7 antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 11.

37. The formulation of claim 35, wherein the anti-integrin beta7 antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 12.

38. The formulation of any one of claims 35-37, wherein the anti-integrin beta7 antibody is etrolizumab.

39. An article of manufacture comprising the formulation of any one of claims 1-38 and a subcutaneous administration device.

40. The article of manufacture of claim 39, wherein the subcutaneous administration device is a needle safety device.

41. The article of manufacture of claim 39, wherein the needle safety device comprises a prefilled syringe.

42. The article of manufacture of any one of claims 39-41, wherein the anti-integrin beta7 antibody is stable at the subcutaneous administration device for at least about 60 months at 5°C, or at least about 3 months at 25°C.

43. The article of manufacture of claim 41 or 42, wherein the prefilled syringe comprises a glass barrel, a plunger stopper, a needle, and a needle shield or a tip cap.

44. The article of manufacture of claim 43, wherein the needle shield is a rigid needle shield.

45. The article of manufacture of claim 44, wherein the rigid needle shield comprises a rubber formulation having low zinc content.

46. The article of manufacture of claim 44 or 45, wherein the rigid needle shield comprises an elastomeric component, and a rigid shield.
47. The article of manufacture of any one of claims 41-46, wherein the prefilled syringe is assembled into an autoinjector.
48. The article of manufacture of any one of claims 40-46, wherein the prefilled syringe comprises silicone oil.
49. The article of manufacture of claim 48, wherein the amount of silicone oil in the prefilled syringe is not greater than about 1 mg.
50. The article of manufacture of claim 48 or 49, wherein the amount of silicone oil in the prefilled syringe is between about 0.1 mg and about 1 mg.
51. The article of manufacture of any one of claims 48-50, wherein the amount of silicone oil in the prefilled syringe is between about 0.2 mg and about 0.6 mg.
52. The article of manufacture of any one of claims 48-50, wherein the amount of silicone oil in the prefilled syringe is between about 0.5 mg and 0.9 mg.
53. The article of manufacture of any one of claims 40-52, wherein the needle safety device has an injection force that is not greater than about 50 Newton (N).
54. The article of manufacture of any one of claims 40-53, wherein the needle safety device has an injection force that is not greater than about 35 Newton (N).
55. The article of manufacture of any one of claims 39-54, comprising between about 0.5 mL and about 2.0 mL of the formulation.
56. The article of manufacture of any one of claims 39-55, comprising between about 0.5 mL and about 1.0 mL of the formulation.
57. The article of manufacture of any one of claims 39-56, comprising about 1.0 mL of the formulation.
58. The article of manufacture of any one of claims 39-56, comprising about 0.7 mL of the formulation.

59. The article of manufacture of any one of claims 39-55, comprising between about 1.0 mL and about 1.5 mL of the formulation.

60. The article of manufacture of any one of claims 39-55 and 59, comprising about 1.4 mL of the formulation.

61. The article of manufacture of any one of claims 41-60, wherein the prefilled syringe has a syringe capacity of 1 mL.

62. The article of manufacture of any one of claims 41-60, wherein the prefilled syringe has a syringe capacity of 2.25 mL.

63. An article of manufacture comprising about 0.7 mL of a formulation and a subcutaneous administration device, wherein

(a) the formulation comprises an anti-integrin beta7 antibody, in 20 mM histidine buffer or about 20 mM histidine buffer, pH 5.8 or pH between 5.7 and 5.9 or pH between 5.75 and 5.85, 0.04% polysorbate 20 or about 0.04% polysorbate 20, and 200 mM arginine succinate or about 200 mM arginine succinate, and wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

(i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;

(ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO:2;

(iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3;

(iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4;

(v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and

(vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or SEQ ID NO:7, and

(b) the subcutaneous administration device is a needle safety device that comprises 1 mL of a prefilled syringe with a syringe capacity of 1 mL.

64. The article of manufacture of claim 63, wherein the anti-integrin beta7 antibody is present in the formulation at a concentration of 150 mg/mL or about 150 mg/mL.

65. An article of manufacture comprising about 1.4 mL of a formulation and a subcutaneous administration device, wherein

(a) the formulation comprises an anti-integrin beta7 antibody, in 20 mM histidine buffer or about 20 mM histidine buffer, pH 5.8 or pH between 5.7 and 5.9 or pH between 5.75 and 5.85, 0.04% polysorbate 20 or about 0.04% polysorbate 20, and 200 mM arginine succinate or about 200 mM arginine succinate, and wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

(i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;

(ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO:2;

(iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3;

(iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4;

(v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and

(vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or SEQ ID NO:7, and

(b) the subcutaneous administration device is a needle safety device that comprises 1 mL of a prefilled syringe with a syringe capacity of 2.25 mL.

66. The article of manufacture of claim 65, wherein the anti-integrin beta7 antibody is present in the formulation at a concentration of 150 mg/mL or about 150 mg/mL.

67. An autoinjector comprising the article of manufacture of any one of claims 39-66.

68. An autoinjector comprising an article of manufacture comprising about 0.7 mL of a formulation and a subcutaneous administration device, wherein

(a) the formulation comprises an anti-integrin beta7 antibody, in 20 mM histidine buffer or about 20 mM histidine buffer, pH 5.8 or pH between 5.7 and 5.9 or pH between

5.75 and 5.85, 0.04% polysorbate 20 or about 0.04% polysorbate 20, and 200 mM arginine succinate or about 200 mM arginine succinate, and wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

- (i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;
- (ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID NO:2;
- (iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID NO:3;
- (iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID NO:4;
- (v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and
- (vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or SEQ ID NO:7, and

(b) the subcutaneous administration device is a needle safety device that comprises 1 mL of a prefilled syringe with a syringe capacity of 1 mL.

69. The autoinjector of claim 68, wherein the anti-integrin beta7 antibody is present in the formulation at a concentration of 150 mg/mL or about 150 mg/mL.

70. An autoinjector comprising article of manufacture comprising about 1.4 mL of a formulation and a subcutaneous administration device, wherein

(a) the formulation comprises an anti-integrin beta7 antibody, in 20 mM histidine buffer or about 20 mM histidine buffer, pH 5.8 or pH between 5.7 and 5.9 or pH between 5.75 and 5.85, 0.04% polysorbate 20 or about 0.04% polysorbate 20, and 200 mM arginine succinate or about 200 mM arginine succinate, and wherein the anti-integrin beta7 antibody comprises three light chain hypervariable regions (HVRs), HVR-L1, HVR-L2, and HVR-L3, and three heavy chain HVRs, HVR-H1, HVR-H2, and HVR-H3, wherein:

- (i) the HVR-L1 comprises the amino acid sequence set forth in SEQ ID NO:1;
- (ii) the HVR-L2 comprises the amino acid sequence set forth in SEQ ID

NO:2;

(iii) the HVR-L3 comprises the amino acid sequence set forth in SEQ ID

NO:3;

(iv) the HVR-H1 comprises the amino acid sequence set forth in SEQ ID

NO:4;

(v) the HVR-H2 comprises the amino acid sequence SEQ ID NO:5; and

(vi) the HVR-H3 comprises the amino acid sequence set forth in SEQ ID NO:6 or SEQ ID NO:7, and

(b) the subcutaneous administration device is a needle safety device that comprises 1 mL of a prefilled syringe with a syringe capacity of 2.25 mL.

71. The autoinjector of claim 70, wherein the anti-integrin beta7 antibody is present in the formulation at a concentration of 150 mg/mL or about 150 mg/ml.

72. A method of treating a gastrointestinal inflammatory disorder in a subject, comprising administering to the subject an effective amount of the formulation of any one of claims 1-38.

73. The method of claim 72, wherein the gastrointestinal inflammatory disorder is an inflammatory bowel disease.

74. The method of claim 73, wherein the inflammatory bowel disease is ulcerative colitis or Crohn's disease.

75. A method of administering subcutaneously a formulation comprising an anti-integrin beta7 antibody, comprising administering subcutaneously the article of manufacture of any one of claims 39-66 or the autoinjector of any one of claims 67-71.

76. The method of claim 75, wherein the administration results in mild pain or no pain.

77. The method of claim 75 or 76, wherein the administration results in a transient and mild injection site reaction.

78. The method of any one of claims 75-77, wherein the full dose is administered or at least 90% of the full dose is administered.

79. The method of any one of claims 75-78, wherein the administration provides an equivalent exposure to etrolizumab compared to a prefilled syringe with a needle safety device.
80. The method of any one of claims 75-79, comprising the formulation of any one of claims 1-37.
81. The formulation of any one of claims 1-38 for use in a therapy.
82. The article of manufacture of any one of claims 39-66 for use in a therapy.
83. The autoinjector of any one of claims 67-71 for use in a therapy.
84. The formulation of any one of claims 1-38, article of manufacture of any one of claims 39-66, or autoinjector of any one of claims 67-71 for use in treating a gastrointestinal inflammatory disorder in a subject.
85. The formulation, article of manufacture, or autoinjector for use of claim 84, wherein the gastrointestinal inflammatory disorder is an inflammatory bowel disease.
86. The formulation, article of manufacture, or autoinjector for use of claim 85, wherein the inflammatory bowel disease is ulcerative colitis or Crohn's disease.