

Description

Title of Invention

FORMULATION FOR ANTI-FCRN ANTIBODY

Technical Field

The present invention relates to a formulation optimized for HL161BKN, which is an anti-FcRn antibody.

Background Art

The causes of autoimmune diseases have been studied for a long time from genetic, environmental and immunological perspectives, but the exact cause of the disease is still unknown. Many recent studies have revealed that a number of autoimmune diseases are caused by IgG-type autoantibodies. In fact, in studies for the diagnosis and treatment of autoimmune diseases, the relationship between the presence or absence of disease-specific autoantibodies and the therapeutic effect according to the reduction thereof has been widely investigated.

As a therapeutic agent for such autoimmune diseases, the first-line agent is a systemic high-dose steroid injection, and when symptoms are serious or difficult to control with steroids, high-dose IVIG (intravenous immunoglobulin) administration or plasmapheresis is applied. High-dose steroid may have a weak effect or have serious side effects when repeatedly used. In the case of IVIG and plasmapheresis, the cost of treatment is high and there are various side effects and risks of infection, so the development of a therapeutic agent in this treatment field is urgently needed.

On the other hand, recently, a therapeutic agent for autoimmune disease using an FcRn antibody is being studied (Korean Patent Publication No. 10-2014-0147606). It is a drug with a new strategy, in which the antibody blocks FcRn (Neonatal Fc Receptor), which is involved in the recycling of IgG, and increases the catabolism of IgG in the body, thereby lowering the levels of autoantibodies to treat the diseases. This anti-FcRn antibody is expected as a product that can solve the problems of existing therapeutic agents.

However, in order to apply this antibody to severe autoimmune diseases such as pemphigus vulgaris, neuromyelitis optica, and myasthenia gravis, which are caused by the

generation of an autoantibody against an autoantigen in the body, a formulation optimized for this antibody and a formulation optimized for the administration method are required.

Prior Art Document

Patent Document

(Patent Document 1) KR 10-2014-0147606 A

Disclosure of Invention

Technical Problem

As a result of research in order to develop a formulation optimized for an anti-FcRn antibody for treating severe autoimmune diseases, the present inventors developed a buffer and formulation optimized for HL161BKN, which is an anti-FcRn antibody.

Solution to Problem

In order to achieve the above object, in one aspect of the present invention, there is provided a pharmaceutical formulation comprising (a) an anti-FcRn antibody or a fragment thereof, (b) at least one additive selected from mannitol, sorbitol, arginine, histidine, glycine and salts thereof, (c) a buffer system selected from citrate or histidine, and (d) a surfactant.

Effects of Invention

A pharmaceutical formulation according to the present invention having a pH of 4.0 to 8.0, comprising (a) an anti-FcRn antibody or a fragment thereof, (b) at least one additive selected from mannitol, sorbitol, arginine, histidine, glycine and salts thereof, (c) a buffer system selected from citrate or histidine, and (d) a surfactant, is a pharmaceutical composition optimized for HL161BKN, and it was confirmed that the stability of HL161BKN in the formulation was improved.

Brief Description of Drawings

Figure 1 illustrates a result obtained by analyzing the thermal stability of HL161BKN using DSC (Differential Scanning Calorimetry).

Figure 2 illustrates a result obtained by confirming the 4-week accelerated stability of

HL161BKN under conditions of sodium citrate-phosphate buffer at pH 5.0, 6.0, 7.0, and 8.0.

Figure 3 is a schematic diagram of the process for studying the formulation of HL161BKN.

Figure 4 illustrates a result obtained by confirming the amount of change in aggregates and fragments of HL161BKN (210 mg/mL) produced according to the various excipients as a result of excipient test.

Figure 5 illustrates a result obtained by analyzing the pattern of charge variants of HL161BKN (210 mg/mL) according to each excipient condition using CEX-HPLC.

Figure 6 illustrates a result of analysis of variance (ANOVA) of the amount of change in aggregates of HL161BKN according to the combination of excipients.

Figure 7 illustrates a result obtained by confirming the amount of change in aggregates and fragments of HL161BKN (210 mg/mL) produced according to the combination of excipients.

Figure 8 illustrates a result obtained by analyzing the amount of change in aggregates and fragments of HL161BKN according to the combination of excipients using Design of Experiment (DOE).

Figure 9 illustrates a result obtained by analyzing the pattern of charge variants of HL161BKN according to the combination of excipients using CEX-HPLC.

Figure 10 illustrates a result obtained by confirming the amount of change in aggregates and fragments of HL161BKN (210 mg/mL) in the additional excipient test.

Figure 11 illustrates a result obtained by analyzing the pattern of charge variants of HL161BKN according to the excipient conditions in the additional excipient test using CEX-HPLC.

Figure 12 illustrates a result obtained by confirming the effect of HL161BKN (210 mg/mL) on agitation stress according to the presence or absence of PSB20.

Figure 13 illustrates a result obtained by confirming the change in the monomer of HL161BKN according to the presence or absence of PSB20 in the agitation test.

Figure 14 illustrates a result obtained by confirming the amount of change in aggregates and fragments of HL161BKN (210 mg/mL) in the viscosity reducing excipient test.

Figure 15 illustrates a result obtained by confirming the change in aggregates and fragments of HL161BKN (210 mg/mL) in the first excipient screening.

Figure 16 illustrates a result obtained by analyzing the pattern of charge variants of

HL161BKN according to the first excipient screening conditions using CEX-HPLC.

Figure 17 illustrates a result obtained by confirming the change in aggregates and fragments of HL161BKN (210 mg/mL) in the second excipient screening.

Figure 18 illustrates a result obtained by analyzing the pattern of charge variants of HL161BKN according to the second excipient conditions using CEX-HPLC.

Figure 19 illustrates a result obtained by confirming the change in the number of insoluble sub-visible particles of HL161BKN.

Figure 20 illustrates a result obtained by confirming the viscosity according to the concentration of HL161BKN in the HL161BKN formulation. Here, when HL161BKN was at a concentration of 170 mg/mL, the viscosity was confirmed to be 10 cP.

Detailed Description for Carrying out the Invention

In one aspect of the present invention, there is provided a pharmaceutical formulation having a pH of 4.0 to 8.0, comprising (a) an anti-FcRn antibody or a fragment thereof, (b) at least one additive selected from mannitol, sorbitol, arginine, histidine, glycine and salts thereof, (c) a buffer system selected from citrate or histidine, and (d) a surfactant.

In addition, the pharmaceutical formulation may further comprise methionine. In addition, the pharmaceutical formulation may further comprise saccharides such as sucrose and trehalose.

The buffer system refers to a buffer that is resistant to changes in pH due to its acid-base counterpart.

As used herein, the term “histidine buffer” refers to a buffer containing a histidine ion. Here, specific embodiments of the histidine buffer may be any one selected from the group consisting of a histidine chloride buffer, a histidine acetate buffer, a histidine phosphate buffer, and a histidine sulfate buffer, but are not limited thereto.

As used herein, the term “citrate buffer” refers to a buffer containing a citrate ion. Here, specific embodiments of the citrate buffer may be any one selected from the group consisting of a sodium citrate buffer, a potassium citrate buffer, a calcium citrate buffer, and a magnesium citrate buffer, but are not limited thereto.

As used herein, the term “surfactant” refers to a pharmaceutically acceptable excipient that is used to protect a protein formulation against mechanical stress such as agitation and

shearing. Specific embodiments of the surfactant may be any one surfactant selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer, Triton, sodium dodecyl sulfate, sodium lauryl sulfonate, sodium octyl glycoside, lauryl-sulfobetaine, myristyl-sulfobetaine, linoleyl-sulfobetaine, stearyl-sulfobetaine, lauryl-sarcosine, myristyl-sarcosine, linoleyl-sarcosine, stearyl-sarcosine, linoleyl-betaine, myristyl-betaine, cetyl-betaine, lauryl amidopropyl-betaine, cocamidopropyl-betaine, linoleamidopropyl-betaine, myristamidopropyl-betaine, palmitoylamidopropyl-betaine, isostearamidopropyl-betaine, myristamidopropyl-dimethylamine, palmitoylamidopropyl-dimethylamine, isostearamidopropyl-dimethylamine, sodium methyl cocacyl, sodium methyl oleyl-taurate, polyethylene glycol, polypropylene glycol, and copolymer of ethylene and propylene glycol. Here, the surfactant may be preferably polysorbate 20.

In addition, the pharmaceutical formulation may be an aqueous formulation, preferably an injectable liquid formulation.

Here, the anti-FcRn antibody may be HL161BKN.

The HL161BKN comprises a heavy chain variable region comprising H-CDR1 having the amino acid of SEQ ID NO: 5, H-CDR2 having the amino acid of SEQ ID NO: 6, and H-CDR3 having the amino acid of SEQ ID NO: 7, and a light chain variable region comprising L-CDR1 having the amino acid of SEQ ID NO: 8, L-CDR2 having the amino acid of SEQ ID NO: 9, and L-CDR3 having the amino acid of SEQ ID NO: 10.

In addition, the HL161BKN may comprise the heavy chain and light chain regions shown in Table 1 below. In addition, the heavy chain and light chain may be encoded by the nucleic acids shown in Table 2.

[Table 1]

Item	Amino acid sequence	SEQ ID NO
Heavy chain	QLLQLQESGPG LVKPSETLSL TCTVSGGSLS SSFSYWVWIR QPPGKGLEWI GTIYYSGNTY YNPSLKSRLT ISVDTSKNHF SLKLSSVTAA DTAVYYCARR AGILTGYLDS WGQGTLLVTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKRVE PKSCDKTHTC PPCAPEAAG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPP VLDSGDSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG	1
Light chain	SYVLTQSPSV SVAPGQTARI TCGGNNIGSK SVHWYQQKPG QAPVLVVYDD SDRPSGIPER FSASNSGNTA TLTISRVEAG DEADYYCQVW DSSSDHVVFG GGTKLTVLGQ PKAAPSVTLF PPSSEELQAN KATLVCLISD FYPGAVTVAW KADSSPVKAG VETTTPSKQS NNKYAASSYL SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP TECS	2

The glycosylation sites of the antibody are as follows: Asn301, N-glycan (G0F, G1F, G0-GlcNac, Man5).

[Table 2]

Item	Nucleotide sequence	SEQ ID NO
polynucleotide coding Heavy chain	CAG CTG CTG CTG CAA GAA TCC GGC CCT GGC CTG GTG AAA CCC TCC GAG ACA CTG TCC CTG ACC TGC ACC GTG TCC GGC GGC TCC CTG TCC TCC AGC TTC TCC TAC TGG GTC TGG ATC CGG CAG CCC CCT GGC AAG GGC CTG GAA TGG ATC GGC ACC ATC TAC TAC TCC GGC AAC ACC TAC TAC AAC CCC AGC CTG AAG TCC CGG CTG ACC ATC TCC GTG GAC ACC TCC AAG AAC CAC TTC AGC CTG AAG CTG TCC TCC GTG ACC GCC GCT GAC ACC GCC GTG TAC TAC TGT GCC AGA AGG GCC GGC ATC CTG ACC GGC TAC CTG GAC TCT TGG GGC CAG GGC ACC CTG GTG ACA GTG TCC TCC GCC TCC ACC AAG GGC CCC TCC GTG TTC CCT CTG GCC CCC TCC AGC AAG TCC ACC TCT GGC GGC ACC GCT GCC CTG GGC TGT CTG GTG AAA GAC TAC TTC CCC GAG CCC GTG ACC GTG TCC TGG AAC TCT GGC GCC CTG ACC TCC GGC GTG CAC ACC TTC CCT GCC GTG CTG CAG TCC TCC GGC CTG TAC TCC CTG TCC AGC GTG GTG ACC GTG CCC TCC AGC TCT CTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAC CAC AAG CCC TCC AAC ACC AAG GTG GAC AAG CGG GTG GAA CCC AAG TCC TGC GAC AAG ACC CAC ACC TGT CCC CCC TGT CCT GCC CCT GAA GCT GCT GGC GGC CCT AGC GTG TTC CTG TTC CCC CCA AAG CCC AAG GAC ACC CTG ATG ATC TCC CGG ACC CCC GAA GTG ACC TGC GTG GTG GTG GAC GTG TCC CAC GAG GAC CCT GAA GTG AAG TTC AAT TGG TAC GTG GAC GGC GTG GAA GTG CAC AAC GCC AAG ACC AAG CCC AGA GAG GAA CAG TAC AAC TCC ACC TAC CGG GTG GTG TCC GTG CTG ACC GTG CTG CAC CAG GAC TGG CTG AAC GGC AAA GAG TAC AAG TGC AAG GTC TCC AAC AAG GCC CTG CCT GCC CCC ATC GAA AAG ACC ATC TCC AAG GCC AAG GGC CAG CCC CGC GAG CCC CAG GTG TAC ACA CTG CCC CCT AGC CGG GAA GAG ATG ACC AAG AAC CAG GTG TCC CTG ACA TGC CTG GTG AAG GGC TTC TAC CCC TCC GAC ATT GCC GTG GAA TGG GAG TCC AAC GGC CAG CCC GAG AAC AAC TAC AAG ACC ACC CCC CCT GTG CTG GAC TCC GAC GGC TCA TTC TTC CTG TAC TCC AAG CTG ACC GTG GAC AAG TCC CGG TGG CAG CAG GGC AAC GTG TTC TCC TGC TCC GTG ATG CAC GAG GCC CTG CAC AAC CAC TAC ACC CAG AAG TCC CTG TCC CTG AGC CCC GGC	3
polynucleotide coding light chain	TCT TAC GTG CTG ACC CAG TCC CCC TCC GTG TCC GTG GCT CCT GGC CAG ACC GCC AGA ATC ACC TGT GGC GGC AAC AAC ATC GGC TCC AAG TCC GTG CAC TGG TAT CAG CAG AAG CCC GGC CAG GCC CCC GTG CTG GTG GTG TAC GAC GAC TCC GAC CGG CCC TCT GGC ATC CCT GAG CGG TTC TCC GCC TCC AAC TCC GGC AAC ACC GCC ACC CTG ACC ATC TCC AGA GTG GAA GCC GGC GAC GAG GCC GAC TAC TAC TGC CAA GTG TGG GAC TCC TCC TCC GAC CAC GTG GTG TTC GGC GGA GGC ACC AAG CTG ACC GTG CTG GGC CAG CCT AAG GCC GCT CCC TCC GTG ACC CTG TTC CCC CCA TCC TCC GAG GAA CTG CAG GCC AAC AAG GCC ACC CTG GTC TGC CTG ATC TCC GAC TTC TAC CCT GGC GCC GTG ACC GTG GCC TGG AAG GCC GAC AGC TCT CCT GTG AAG GCC GGC GTG GAA ACC ACC ACC CCC TCC AAG CAG TCC AAC AAC AAA TAC GCC GCC TCC TCC TAC CTG TCC CTG ACC CCC GAG CAG TGG AAG TCC CAC CGG TCC TAC AGC TGC CAA GTG ACA CAC GAG GGC TCC ACC GTG GAA AAG ACC GTG GCC CCT ACC GAG TGC TCC	4

Here, the pharmaceutical formulation may have a viscosity of 20 cP or less. Specifically, the pharmaceutical formulation may have a viscosity of 1 cP to 20 cP. In addition, the pharmaceutical formulation may have a viscosity of 10 cP to 20 cP, and may have a viscosity of about 10 cP, about 11 cP, about 12 cP, about 13 cP, about 14 cP, about 15 cP, about 16 cP, about 17 cP, about 18 cP, about 19 cP, or about 20 cP.

In addition, the pharmaceutical formulation may have an osmolality of 250 mOs/kg to 500 mOs/kg. Specifically, the pharmaceutical formulation may have an osmolality of 300 mOs/kg to 450 mOs/kg or 350 mOs/kg to 400 mOs/kg. In addition, the pharmaceutical formulation may have an osmolality of about 250 mOs/kg, about 260 mOs/kg, about 270 mOs/kg, about 280 mOs/kg, about 290 mOs/kg, about 300 mOs/kg, about 310 mOs/kg, about 320 mOs/kg, about 330 mOs/kg, about 340 mOs/kg, about 350 mOs/kg, about 360 mOs/kg, about 370 mOs/kg, about 380 mOs/kg, about 390 mOs/kg, about 400 mOs/kg, about 410 mOs/kg, about 420 mOs/kg, about 430 mOs/kg, about 440 mOs/kg, about 450 mOs/kg, about 460 mOs/kg, about 470 mOs/kg, about 480 mOs/kg, about 490 mOs/kg, or about 500 mOs/kg.

In addition, the pharmaceutical formulation may include HL161BKN at a concentration of 50 mg/mL to 250 mg/mL. Specifically, the pharmaceutical formulation may include HL161BKN at a concentration of 60 mg/mL to 250 mg/mL, 70 mg/mL to 250 mg/mL, 80 mg/mL to 250 mg/mL, 90 mg/mL to 250 mg/mL, or 100 mg/mL to 250 mg/mL. In addition, the pharmaceutical formulation may include HL161BKN at a concentration of 120 mg/mL to 230 mg/mL, 150 mg/mL to 220 mg/mL, or 180 mg/mL to 210 mg/mL. In addition, the pharmaceutical formulation may include HL161BKN at a concentration of about 50 mg/mL, about 60 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 110 mg/mL, about 120 mg/mL, about 130 mg/mL, about 140 mg/mL, about 150 mg/mL, about 160 mg/mL, about 170 mg/mL, about 180 mg/mL, about 190 mg/mL, about 200 mg/mL, about 210 mg/mL, about 220 mg/mL, about 230 mg/mL, about 240 mg/mL, or about 250 mg/mL.

In addition, the pharmaceutical formulation may have a pH of 4.0 to 8.0. Specifically, the pharmaceutical formulation may have a pH of 4.0 to 7.0. Preferably, the pharmaceutical formulation may have a pH of 5.0 to 6.0. In addition, the pharmaceutical formulation may have about pH 5.0, about pH 5.1, about pH 5.2, about pH 5.3, about pH 5.4, about pH 5.5, about pH 5.6, about pH 5.7, about pH 5.8, about pH 5.9, about pH 6.0, about pH 6.1, about pH 6.2, about pH 6.3, about pH 6.4, about pH 6.5, about pH 6.6, about pH 6.7, about pH 6.8, about pH 6.9, or

about pH 7.0.

In addition, the additive may be included at a concentration of 10 mM to 400 mM. Here, as the additive, mannitol, sorbitol, arginine, histidine or glycine may be used alone, and two or more may be used in combination. Specifically, the additive may be included at a concentration of 10 mM to 400 mM, 20 mM to 300 mM, 50 mM to 250 mM, or 100 mM to 150 mM, respectively. Specifically, the additive may be included at a concentration of about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, about 100 mM, about 110 mM, about 120 mM, about 130 mM, about 140 mM, about 150 mM, about 160 mM, about 170 mM, about 180 mM, about 190 mM, about 200 mM, about 210 mM, about 220 mM, about 230 mM, about 240 mM, about 250 mM, about 260 mM, about 270 mM, about 280 mM, about 290 mM, or about 300 mM, respectively.

In addition, two of the additives may be used in combination. In one embodiment, the additive may include mannitol and sorbitol. In one embodiment, the additive may include mannitol and arginine. In one embodiment, the additive may include mannitol and histidine. In one embodiment, the additive may include mannitol and glycine. In one embodiment, the additive may include sorbitol and arginine. In one embodiment, the additive may include sorbitol and histidine. In one embodiment, the additive may include sorbitol and glycine. In one embodiment, the additive may include arginine and histidine. In one embodiment, the additive may include arginine and glycine. In one embodiment, the additive may include histidine and glycine. Here, each additive may be included in a pharmaceutical formulation at the above-mentioned concentration.

One embodiment of the pharmaceutical formulation may be a pharmaceutical formulation having a pH of 5.0 to 6.0, comprising (a) 100 mg/mL to 250 mg/mL of an anti-FcRn antibody, (b) 50 to 250 mM L-arginine or a hydrochloride salt thereof, (c) 50 to 250 mM L-histidine, and (d) 0.01 to 0.05% polysorbate 20.

Here, the anti-FcRn antibody is as described above. In addition, the pharmaceutical formulation may be an aqueous formulation, and may be an injectable liquid formulation. In addition, the above-mentioned pharmaceutical formulation may be administered subcutaneously.

In addition, it was confirmed that the pharmaceutical formulation is very stable under accelerated conditions. Specifically, the content of aggregates and fragments may be about 10% or less as a result of a 6-month test under an accelerated condition (25°C, a relative humidity of

60%). In addition, the content of aggregates and fragments under the above condition may be about 9% or less, about 8% or less, about 7% or less, about 6% or less, about 5.5% or less, or about 5.0% or less.

In addition, it was confirmed that the pharmaceutical formulation is very stable even under a long-term storage condition. Specifically, the content of aggregates and fragments under the condition of 5°C and 36 months may be about 10% or less. In addition, the content of aggregates and fragments under the above condition may be about 9% or less, about 8% or less, about 7% or less, about 6% or less, about 5% or less, about 4% or less, about 3% or less, about 2% or less, about 1.8% or less, about 1.5% or less, or about 1.2% or less.

In addition, the pharmaceutical formulation may be used for the treatment of autoimmune diseases. Here, the autoimmune disease may be any one selected from the group consisting of myasthenia gravis (MG), thyroid eye disease (TED), warm autoimmune hemolytic anemia (WAIHA), neuromyelitis optica (NMO), immune thrombocytopenic purpura (ITP), pemphigus vulgaris (PV), chronic inflammatory demyelinating polyneuropathy (CIDP), lupus nephritis (LN), and membranous nephropathy (MN).

Screening of formulation optimized for HL161BKN

HL161BKN was prepared at a concentration of about 210 mg/mL for each condition and stored for 4 weeks under accelerated conditions of 40°C, and the analysis of concentration (A280), turbidity (A340), purity (SEC-HPLC, CEX-HPLC), viscosity, osmolality, insoluble sub-visible particle (MFI) and the like was performed to evaluate the stability of the sample and suitability for subcutaneous administration.

First, a screening test was performed on 11 excipients that are frequently used in existing antibody products. As a result, it was confirmed that L-histidine, L-arginine hydrochloride, L-glycine, D-sorbitol, and D-mannitol had the effect of reducing the formation of aggregates.

A synergistic effect was tested through a combination of five selected excipients. As a result, it was confirmed that L-arginine hydrochloride reduced the formation of aggregates at a statistically significant level ($p < 0.01$).

By combining the results of comparison of effect values on aggregates and fragmentation, L-histidine and D-mannitol were selected as excipients. In addition, as a result of screening for additional excipients, L-methionine, which exhibited the effect of reducing the formation of aggregates, was additionally selected. In addition, it was confirmed that histidine

can be used as a basal buffer. In addition, high-purity 0.02% polysorbate 20, which exhibited the effect of inhibiting the formation of aggregates due to agitation stress that may occur during storage and transport of the product, was selected.

Next, the first concentration screening test of the selected histidine basal buffer and L-arginine hydrochloride, D-mannitol, and L-methionine excipients was performed. Here, the concentration of PBS20 was fixed at 0.02% and performed. As a result, high stability was confirmed under conditions of 50 mM histidine basal buffer without excipients. In addition, the amount of aggregates and fragments increased according to the excipient conditions did not show a significant difference, but the formation of aggregates was reduced as the concentration of L-arginine hydrochloride added was increased.

On the other hand, it was confirmed that there was little difference according to the presence or absence of D-mannitol and the concentration of L-methionine. Therefore, L-arginine hydrochloride and 0.02% PSB20 were selected in a L-histidine basal buffer as a HL161BKN formulation.

Finally, in order to select the optimal concentrations of L-histidine and L-arginine hydrochloride, the second concentration screening was performed with two batches of HL161BKN. As a result, the amount of aggregates and fragments increased according to the concentration conditions of the excipient did not show a significant difference, and it was confirmed that the measured values of viscosity and osmolality under all conditions were 20 cP or less and 250 to 500 mOsmol/kg, which were suitable for subcutaneous administration.

In addition, as a result of performing the analysis of insoluble sub-visible particles using micro flow imaging (MFI) for each condition sample, the condition in which the increase in the number of insoluble sub-visible particles was the least was confirmed; and the stability and subcutaneous injection (SC) administration suitability through the purity (aggregates and fragments), viscosity, and osmolality tests were evaluated to select the final formulation.

In conclusion, about 100 mM L-histidine, about 100 mM L-arginine hydrochloride, and about 0.02% polysorbate 20 (pH 6.0) were selected as a formulation for non-clinical and phase 1 clinical trials of HL161BKN.

Hereinafter, the present invention will be described in more detail through the examples. These examples are only for illustrating the present invention, and it will be apparent to those of ordinary skill in the art that the scope of the present invention is not to be construed as being

limited by these examples.

I. Preparation of HL161BKN

Preparation Example 1. Preparation of gene construct and vector containing them for preparing HL161 antibody

In order to prepare HL161BKN, a polynucleotide having the nucleic acid sequence of SEQ ID NO: 3 encoding a heavy chain comprising the amino acid sequence of SEQ ID NO: 1 was loaded into the pCHO 1.0 vector (Life Technologies). In addition, a polynucleotide having the nucleic acid sequence of SEQ ID NO: 4 encoding a light chain comprising the amino acid sequence of SEQ ID NO: 2 was loaded into the pCHO 1.0 vector.

Preparation Example 2. Preparation of HL161BKN cell line and production of HL161BKN

CHO-S cells were transformed using the expression vector prepared in Preparation Example 1, and subjected to the selection process of methotrexate and puromycin, and then a final production cell line was prepared. The prepared cell line was prepared and stored as a cell bank, and used for HL161BKN production. The antibody production was performed in a bioreactor containing a culture medium (Dynamis medium + 8 mM L-glutamine) by adding an additional medium (EFB+) every 2 days, and the supernatant was recovered after culturing for about 15 days. Thereafter, a Protein A column was performed, and viral inactivation was performed at low pH, and then anion exchange chromatography (AEX) and cation exchange chromatography (CEX) were performed. Thereafter, after concentration and buffer exchange (Ultrafiltration/Diafiltration), it was purified by sterile filtration. The quality of the produced antibody sample was confirmed through analysis such as SEC-HPLC, CE-SDS, cIEF, ELISA, potency, and concentration.

Preparation Example 3. Analysis of basic properties of HL161BKN

Thermal stability of HL161BKN, stability test under accelerated conditions according to pH, and solubility test were performed. For thermal stability, T_m values were analyzed by DSC (differential scanning calorimetry), and for accelerated condition experiment for each pH, the degree of the formation of aggregates and fragments was monitored while storing at 37°C for 1 month.

For the DSC for T_m value analysis, the equipment from Osong Hi-tech Medical Industry Promotion Foundation was used. Experiments were conducted in the range of 25°C to 100°C,

and the results were confirmed as shown in Figure 1. A typical DSC histogram of the IgG1 type was shown, and curves according to the CH domain and Fv domain were confirmed. HL161BKN exhibited a high T_m of 79.9°C and was analyzed to be thermodynamically very stable.

In addition, using sodium citrate-phosphate buffers at pH 5.0, 6.0, 7.0, and 8.0, HL161BKN was prepared at a concentration of 10 mg/mL. Thereafter, while storing at 37°C for 4 weeks, the degree of the formation of aggregates or fragments was confirmed using SEC-HPLC. As a result, the formation of aggregates or fragments was increased as the pH was increased (Figure 2).

Based on this result, HL161BKN was prepared at a concentration of 100 mg/mL using buffers having a low concentration or high concentration, which is low pH of 5.0 and 6.0, and then the stability test was performed while storing for 4 weeks under accelerated conditions of 40°C. As a result, HL161BKN exhibited a tendency to be stable under low pH conditions, pH 5.0 to 6.0, and maintained a high level of monomer overall under all conditions.

In addition, in order to confirm the solubility of HL161BKN, it was concentrated in 6 steps to a concentration of 10 to 300 mg/mL, and samples were collected step by step and visually observed, and the analysis of A280 nm (concentration), A340 nm (turbidity), and SEC-HPLC purity was performed. As a result, it was confirmed that the turbidity of HL161BKN was gradually increased as the concentration thereof was increased, but there was little change in the monomer purity at a concentration of up to 268 mg/mL.

II. Selection of formulation optimized for HL161BKN

Preparation Example 1. Screening of formulation optimized for HL161BKN

The purpose of this experiment is to select the formulation of the drug substance and drug product for the development of high concentration subcutaneous administration products of HL161BKN. In this experiment, three production batches of HL161BKN-B005, HL161BKN-B018, and HL161BKN-B021 were used, and the reagents and instruments used are as follows (Table 3).

[Table 3]

Components	Manufacturer
D-(+)-Trehalose dihydrate	Sigma
D-Mannitol	Sigma
D-Mannitol	Avantor
D-Sorbitol	Sigma
L-Arginine monohydrochloride	Merck
L-Histidine monohydrochloride monohydrate	Merck
L-Histidine	Sigma
L-Histidine	Avantor
Polysorbate 20 (PSB20)	Sigma
Polysorbate 20 (PSB20)	Avantor
Polysorbate 80 (PSB80)	Merck
Sodium chloride	Merck
Sucrose	Bioshop
Sucrose	Avantor
L-Glycine	Merck
L-Methionine	Avantor
L-Lysine-monohydrochloride	Tianjin Tianyao pharmaceuticals
L-Valine	Evonik
Sodium sulfate anhydrous	Deajung
Ammonium chloride	Sigma
Citric acid, anhydrous	Avantor
Citric acid, anhydrous	Merck
Acetonitrile	B&J
Tri-sodium citrate, dihydrate	Merck
Tri-sodium citrate, dihydrate	Avantor
1.5 mL microcentrifuge tube	Axygen
Amicon® ultra centricon, 15 mL	Millipore
Amicon® ultra centricon, 4 mL	Millipore

The instruments used for this formulation test are as follows (Table 4).

[Table 4]

Instruments	Manufacturer	Catalog number
Centrifuge	Gyrogen	1580-MGR
mv-ROC Viscometer	Rheosense	mv-ROC
Osmometer	Gonotec	Osmomat 3000
Temperature & Humidity Chamber	Jeio tech	TH-TG-300
Nanodrop	Thermoscientific	Nanodrop2000
Propac WCX-10	Dionex	054993
TSK gel G3000SWXL	TOSOH	5788
TSK SWXL guard column	TOSOH	8543
Water bath	Daihan	DH.WB000111
Waters alliance HPLC	Waters	E2695 / 2489
MyLab intelli mixer	MyLab	SLRM-3
MFI 5200 particle analyzer	ProteinSimple	4000-015-001
Stat-ease Design-Expert® software	Stat-Ease	Version 7.0

Preparation Example 2. Test method

The formulation selection test for HL161BKN was largely divided into tests of an excipient screening and an excipient concentration screening, and performed. Specifically, an experiment was performed in the same manner as in Figure 3.

Example 1. Excipient screening

Example 1.1. Excipient test

Sucrose, D-trehalose, D-mannitol, D-sorbitol, L-arginine HCl, L-histidine HCl, L-histidine, L-glycine, polysorbate 20, polysorbate 80, and sodium chloride (NaCl), which were 11 excipients that are frequently used in antibody products currently on the market, were selected. Specifically, the excipient test was performed in 12 buffer conditions using 5 mM sodium citrate (pH 6.0) as a basal buffer (Table 5).

HL161BKN was concentrated to 1 mL or less using AMICON® (Cutoff MW. 30,000), and then a buffer was exchanged with the corresponding buffer conditions to obtain a final concentration of 210 mg/mL. Samples for each condition were prepared by 0.3 to 0.5 mL, and stored in 1.5 mL microcentrifuge tubes at 40°C for 4 weeks. Samples were sampled at week 0, week 2, and week 4 to evaluate changes in concentration (A280), turbidity (A340), and purity (SEC-HPLC, CEX-HPLC).

[Table 5]

Buffer	Target concentration (mg/mL)	11 Excipients										
		Sucrose (mM)	D-Trehalose (mM)	D-Mannitol (mM)	D-Sorbitol (mM)	L-Arg-HCl (mM)	L-His-HCl (mM)	L-His (mM)	L-Gly (mM)	PSB 20 (%)	PSB 80 (%)	NaCl (mM)
5 mM Na citrate (pH 6.0)	210	-	-	-	-	-	-	-	-	-	-	-
		250	-	-	-	-	-	-	-	-	-	-
		-	200	-	-	-	-	-	-	-	-	-
		-	-	200	-	-	-	-	-	-	-	-
		-	-	-	250	-	-	-	-	-	-	-
		-	-	-	-	50	-	-	-	-	-	-
		-	-	-	-	-	50	-	-	-	-	-
		-	-	-	-	-	-	20	-	-	-	-
		-	-	-	-	-	-	-	100	-	-	-
		-	-	-	-	-	-	-	-	0.2	-	-
		-	-	-	-	-	-	-	-	-	0.2	-
		-	-	-	-	-	-	-	-	-	-	150

Example 1.2. Excipient combination test

When combining the five excipients (L-histidine, L-arginine hydrochloride, L-glycine, D-sorbitol, and D-mannitol) selected in Example 1.1, it was confirmed whether there was a synergistic effect. Specifically, 17 condition tests were planned in a 2-level factorial (2^{n-1}) design using DOE (design of experiments) software (Stat-ease DESIGN-EXPERT[®], version 7.0) (Table 6). Specifically, a total of 12 condition tests were performed: 5 mM sodium citrate (pH 6.0) basal buffer condition and 11 excipient combination conditions. In addition, the test results of Example 1.1 were used for the five excipients only condition tests.

The test method was performed in the same manner as in Example 1.1, and the osmolality of the buffer and sample for each condition was further measured, and ANOVA analysis and effect values were calculated using the DOE Software.

[Table 6]

Basal Buffer	HL161BKN concentration (mg/mL)	Sample No.	Excipients (mM)				
			His	Arg-HCl	Gly	Sorbitol	Mannitol
5 mM Na citrate (pH 6.0)	210	Control	-	-	-	-	-
		1	50	-	-	-	-
		2	-	50	-	-	-
		3	-	-	100	-	-
		4	-	-	-	250	-
		5	-	-	-	-	200
		6	50	-	100	250	-
		7	50	50	-	250	-
		8	50	50	100	-	-
		9	50	50	-	-	200
		10	50	-	100	-	200
		11	-	50	100	250	-
		12	50	-	-	250	200
		13	-	50	100	-	200
		14	-	-	100	250	200
		15	-	50	-	250	200
		16	50	50	100	250	200

Example 1.3. Test for additional excipient

An additional analysis of excipients for the HL161BKN formulation test was performed.

An additional test of L-methionine, which is known to have the effect of reducing covalent aggregates; and a test of changing the sodium citrate (pH 6.0) basal buffer, which may cause pain during injection, to histidine (pH 6.0) were performed. In addition, when low-quality PSB20 (Polysorbate 20) containing a lot of peroxide is used, the antibody stability is reduced due to oxidation, so it was changed to high-quality PSB20 for formulation. Thereafter, the effect on agitation stress was confirmed at a concentration of 0.02%, which is commonly used in the formulation. Then, an excipient capable of reducing the viscosity of the high concentration antibody product was screened.

Example 1.3.1. Test for L-methionine and histidine basal buffer

In order to confirm the effect of L-methionine and whether the basal buffer, 5 mM sodium citrate (pH 6.0), can be changed to histidine (pH 6.0), a test was performed under the six conditions in Table 7. In the case of the histidine basal buffer, L-histidine and L-histidine

hydrochloride were mixed to prepare them at a pH of 6.0 and used. The test method was performed in the same manner as in Example 1.1.

[Table 7]

HL161BKN concentration (mg/mL)	Conditions	Buffer
210	1	5 mM Na Citrate (pH 6.0)
	2	5 mM Na Citrate, 50 mM Methionine (pH 6.0)
	3	5 mM Na Citrate, 50 mM Histidine (pH 6.0)
	4	5 mM Na Citrate, 0.02% PSB20 (pH 6.0)
	5	10 mM Histidine (pH 6.0)
	6	50 mM Histidine (pH 6.0)

Example 1.3.2. Polysorbate 20 (PSB20) test

In order to confirm the protective effect of PSB20 against agitation stress, a test was performed under the four conditions in Table 8. 0.5 mL of the respective samples prepared in the same manner as in Example 1.1 was placed in a 1.5 mL microcentrifuge tube, and equipped on a MyLab intelli mixer, and rotated at 10 rpm at room temperature for 1 week, and then concentration, turbidity, and purity (SEC-HPLC) analysis was performed.

[Table 8]

Target concentration (mg/mL)	Buffer	PSB20	Agitation
210	5 mM Na citrate (pH6.0)	-	-
		-	+
		0.02%	-
		0.02%	+

Example 1.3.3. Screening of excipient for viscosity reduction

In order to develop HL161BKN high concentration products for subcutaneous administration, the eight excipients, which are generally known to reduce viscosity, were tested. A total of 9 excipient screening tests were performed using 50 mM histidine (pH 6.0) as a basal buffer (Table 9).

Samples were prepared by 1 mL for each condition in the same manner as in Example 1.1, and the viscosity was measured at 25°C. In addition, changes in concentration (A280) and purity (SEC-HPLC) for each condition were evaluated while performing the 4-week accelerated

stability test.

[Table 9]

Basal Buffer	HL161BKN concentration (mg/mL)	Condition	8 Excipients							
			L-His-HCl (mM)	L-Lys-HCl (mM)	L-Arg-HCl (mM)	Gly (mM)	L-Val (mM)	NaCl (mM)	Na ₂ SO ₄ (mM)	NH ₄ Cl (mM)
50 mM Histidine (pH6.0)	210	1	-	-	-	-	-	-	-	-
		2	100	-	-	-	-	-	-	-
		3	-	100	-	-	-	-	-	-
		4	-	-	100	-	-	-	-	-
		5	-	-	-	100	-	-	-	-
		6	-	-	-	-	100	-	-	-
		7	-	-	-	-	-	100	-	-
		8	-	-	-	-	-	-	100	-
		9	-	-	-	-	-	-	-	100

Example 1.4. Excipient concentration screening

Example 1.4.1. First excipient concentration screening

DOE test of 2 level factorial (2ⁿ) design was planned for the 50 mM histidine (pH 6.0) basal buffer selected through Examples 1.1, 1.2 and 1.3 and three excipients (L-methionine, L-arginine hydrochloride, and D-mannitol) of four excipients (L-methionine, L-arginine hydrochloride, D-mannitol, and PSB20), and excipient concentration screening was performed under a total of 9 conditions (Table 10).

The experiment was performed in the same manner as in Example 1.1, and changes in concentration (A280), turbidity (A340), purity (SEC-HPLC, CEX-HPLC), viscosity, and osmolality were analyzed.

[Table 10]

Conditions	L-Met (mM)	L-Arg-HCl (mM)	D-Mannitol (mM)	PSB20 (%)
1	-	-	-	-
2	50	50	0	0.02
3	10	100	0	0.02
4	10	50	100	0.02
5	10	100	100	0.02
6	50	100	100	0.02
7	50	50	100	0.02
8	50	100	0	0.02
9	10	50	0	0.02

Example 1.4.2. Second excipient concentration screening

In order to investigate the optimal concentration of L-arginine hydrochloride among the final selected histidine basal buffer and two excipients (L-arginine hydrochloride and PSB20), a 4-week 40°C accelerated stability test under four conditions was performed using two batches of HL161BKN B018 and B021 (Table 11). The test was performed in the same manner as in Example 1.1, and concentration (A280), turbidity (A340), purity (SEC-HPLC, CEX-HPLC), viscosity, osmolality, and insoluble sub-visible particle (Micro flow imaging; MFI) were analyzed.

[Table 11]

Conditions	L-Histidine (Basal buffer) (mM)	L-Arg-HCl (mM)	PSB20 (%)	HL161BKN Batch#
1	50	100	0.02	B018
2	50	150	0.02	
3	100	100	0.02	
4	150	50	0.02	
1	50	100	0.02	B021
2	50	150	0.02	
3	100	100	0.02	
4	150	50	0.02	

Example 2. Results of Excipient screening test

Example 2.1. Results of Excipient test

In order to evaluate the effect of 11 excipients selected for excipient screening on the

stability of HL161BKN samples, analysis of concentration, turbidity, purity, aggregates, fragments, and charge variants was performed at week 0, week 2, and week 4 under accelerated conditions of 40°C. Through these analysis, 5 excipients (L-arginine hydrochloride, L-histidine, D-mannitol, L-glycine, and D-sorbitol), which had a good effect of inhibiting the formation of aggregates and fragments, were selected.

Example 2.1.1. Results of concentration (A280) and turbidity (A340) analysis

HL161BKN concentration was increased for 4 weeks (Table 12), which was assumed to be due to buffer evaporation under accelerated conditions of 40°C.

[Table 12]

Excipient	0 Week				2 Week				4 Week			
	Meas. OD340	Average	Dilution factor	Concentration	Meas. OD340	Average	Dilution factor	Concentration	Meas. OD340	Average	Dilution factor	Concentration
-	37.090	37.338	10	231.9	41.560	41.567	10	258.2	41.000	40.830	10	253.6
	37.435				41.547				40.747			
	37.489				41.594				40.744			
Histidine	34.625	34.887	10	216.7	38.327	38.761	10	240.7	37.347	37.508	10	233.0
	34.901				38.999				37.580			
	35.136				38.956				37.596			
Histidine HCl	35.391	35.220	10	218.8	35.938	35.735	10	222.0	38.122	38.222	10	237.4
	35.050				35.559				38.241			
	35.220				35.709				38.304			
Arginine HCl	33.643	33.813	10	210.0	36.255	36.215	10	224.9	38.682	39.004	10	242.3
	33.697				36.278				39.231			
	34.098				36.113				39.100			
Trehalose	33.000	32.923	10	204.5	33.704	33.671	10	209.1	34.474	34.704	10	215.6
	33.018				33.627				34.809			
	32.752				33.682				34.829			
Sorbitol	34.590	34.938	10	217.0	36.956	37.086	10	230.3	38.396	38.591	10	239.7
	34.961				37.169				38.726			
	35.262				37.133				38.652			
Sucrose	33.079	33.120	10	205.7	39.543	39.359	10	244.5	39.865	40.004	10	248.5
	33.104				39.384				39.955			
	33.177				39.151				40.193			
Mannitol	34.599	34.561	10	214.7	38.971	38.988	10	242.2	37.017	37.131	10	230.6
	34.613				38.879				37.034			
	34.471				39.114				37.341			
Glycine	33.782	33.608	10	208.7	30.015	30.159	10	187.3	36.080	36.265	10	225.2
	33.296				30.141				36.338			
	33.745				30.320				36.377			
PSB20	36.778	36.623	10	227.5	38.696	39.312	10	244.2	41.305	40.944	10	254.3
	36.524				39.574				40.330			
	36.567				39.666				41.196			
PSB80	32.287	32.284	10	200.5	36.254	36.183	10	224.7	45.823	45.692	10	283.8
	32.445				36.103				45.721			
	32.119				36.193				45.533			
NaCl	30.704	30.708	10	190.7	32.621	32.658	10	202.8	34.538	34.592	10	214.9
	30.679				32.757				34.708			
	30.741				32.597				34.530			

On the other hand, the turbidity was not increased in most conditions, or the amount of change was 0.020 or less. However, it was observed that it was visually cloudy in the PSB20 condition, and it was confirmed that the turbidity (A340) was also greatly increased (Table 13).

[Table 13]

Condition No.	Excipients	Turbidity (A340)			
		Week 0	Week 2	Week 4	ΔA_{340} (W4-W0)
1	-	0.125	0.122	0.145	0.020
2	Sucrose	0.211	0.166	0.167	-0.044
3	D-Trehalose	0.127	0.099	0.098	-0.029
4	D-Mannitol	0.184	0.111	0.113	-0.071
5	D-Sorbitol	0.129	0.106	0.115	-0.014
6	L-Arginine-HCl	0.142	0.095	0.101	-0.041
7	L-Histidine-HCl	0.149	0.108	0.130	-0.019
8	L-Histidine	0.118	0.112	0.124	0.006
9	L-Glycine	0.142	0.100	0.113	-0.029
10	PSB20	0.166	1.159	1.260	1.094
11	PSB80	0.266	0.324	0.275	0.009
12	NaCl	0.162	0.119	0.142	-0.020

Example 2.1.2. Results of aggregate and fragment analysis

SEC-HPLC was used to compare and evaluate the amount of aggregates and fragments increased for each excipient condition (Table 14). Compared with the basal buffer condition, L-arginine hydrochloride, L-histidine, L-histidine hydrochloride, D-mannitol, L-glycine, and D-sorbitol effectively inhibited the formation of aggregates. In addition, 0.2% PSB20, 0.2% PSB80, and NaCl rather increased the formation of aggregates. In addition, L-histidine, L-histidine hydrochloride, L-arginine hydrochloride, and L-glycine were the excipients inhibiting the formation of fragments, and NaCl increased the formation of fragments (Table 14 and Figure 4). [Table 14]

Condition No.	Excipients	Relative purity (monomer)			Changes at week 2 (W2-W0)		Changes at week 4 (W4-W0)	
		Week 0	Week 2	Week 4	Δ Aggre	Δ Frag	Δ Aggre	Δ Frag
1	-	100.0	92.1	84.4	4.3	2.9	10.0	4.3
2	Sucrose	100.0	93.6	87.3	3.3	2.5	7.6	3.9
3	D-Trehalose	100.0	94.9	90.6	2.2	2.5	4.5	4.1
4	D-Mannitol	100.0	95.1	91.5	1.9	2.6	3.6	4.2
5	D-Sorbitol	100.0	94.9	91.4	2.0	2.7	3.8	4.0
6	L-Arginine-HCl	100.0	97.5	96.7	0.2	2.1	0.1	3.0
7	L-Histidine-HCl	100.0	96.1	94.7	2.0	1.7	2.3	2.7
8	L-Histidine	100.0	95.8	94.7	2.0	1.8	2.0	2.5
9	L-Glycine	100.0	96.2	93.2	1.6	1.9	3.6	2.7
10	PSB20	100.0	95.9	84.0	0.9	2.8	10.5	4.0
11	PSB80	100.0	83.5	81.5	12.5	2.4	13.0	3.7
12	NaCl	100.0	86.6	70.7	8.2	4.0	20.1	6.5

Example 2.1.3. Results of charge variant analysis

The pattern of changes in charge variants of HL161BKN according to each excipient condition was confirmed using CEX-HPLC. As a result, no distinct changes in charge variants could be observed. However, the main peak was greatly reduced in PSB20 and NaCl conditions (Figure 5). This was expected as a result of increased aggregates.

Example 2.2. Results of excipient combination test

The effect of the combination of five excipients selected in the excipient test of Example 1.1 on stability of the HL161BKN sample was evaluated. Specifically, the 40°C 4-week accelerated test was performed, and L-arginine hydrochloride, L-histidine, and D-mannitol, which effectively inhibited the formation of aggregates and fragments, were selected.

In order to confirm whether the excipients selected through the excipient screening test have a synergistic effect through combination, a condition test was planned using design of experiments (DOE) software. A total of 12 condition tests were performed, including a basal buffer condition and 11 excipients combination conditions. The test results of Example 1.1 were used for the five excipients alone condition test (Table 15).

When the results of comparison of the amount of change in aggregates were analyzed by ANOVA, an excipient that reduced the formation of aggregates at a statistically significant level ($p < 0.01$) was found. However, there were no excipients that reduced the formation of fragments

at a statistically significant level.

On the other hand, in the case of HL161BKN, the formation pattern of aggregates was different depending on the combination of excipients, but there was no synergistic effect by the combination of excipients. The kinds of excipient was selected with reference to the ANOVA analysis and the comparison ranking of effect values.

[Table 15]

Condition No.	L-Histidine	L-Arg HCl	L-Glycine	D-Sorbitol	D-Mannitol	Test
	50 mM	50 mM	100 mM	250 mM	200 mM	
1	-1	-1	-1	-1	1	X
2	1	-1	1	1	-1	O
3	1	1	-1	1	-1	O
4	1	-1	-1	-1	-1	X
5	1	1	1	-1	-1	O
6	-1	1	-1	-1	-1	X
7	-1	-1	-1	1	-1	X
8	1	1	-1	-1	1	O
9	1	-1	1	-1	1	O
10	1	1	1	1	1	O
11	1	-1	-1	1	1	O
12	-1	1	1	-1	1	O
13	-1	-1	1	1	1	O
14	-1	1	1	1	-1	O
15	-1	1	-1	1	1	O
16	-1	-1	1	-1	-1	X
17	-1	-1	-1	-1	-1	O

Example 2.2.1. Results of concentration (A280) and turbidity (A340) analysis

The concentration of HL161BKN was increased for 4 weeks, which was assumed to be due to buffer evaporation under accelerated conditions of 40°C (Table 16). On the other hand, turbidity was not increased or showed a slight increase to 0.054 or less (Table 17).

[Table 16]

Conditions	0 Week				2 Week				4 Week			
	Meas. OD280	Average	Dilution factor	Concentration	Meas. OD280	Average	Dilution factor	Concentration	Meas. OD280	Average	Dilution factor	Concentration
2	36.559	36.471	10	226.5	36.286	36.333	10	225.7	38.797	39.513	10	245.4
	36.505				36.411				39.767			
	36.349				36.301				39.976			
3	34.230	34.367	10	213.5	37.192	37.215	10	231.2	37.316	37.342	10	231.9
	34.393				37.254				37.534			
	34.477				37.200				37.177			
5	35.852	35.860	10	222.7	38.200	38.062	10	236.4	37.938	37.988	10	236.0
	35.768				38.003				37.875			
	35.961				37.984				38.151			
8	33.291	33.364	10	207.2	36.967	37.044	10	230.1	38.593	38.709	10	240.4
	33.395				37.064				38.919			
	33.406				37.102				38.616			
9	34.744	34.872	10	216.6	36.676	36.908	10	229.2	37.359	37.306	10	231.7
	34.994				37.078				37.507			
	34.877				36.971				37.053			
10	35.955	35.986	10	223.5	36.084	36.158	10	224.6	37.998	38.015	10	236.1
	36.051				36.124				37.981			
	35.952				36.265				38.067			
11	34.664	34.419	10	213.8	36.009	36.020	10	223.7	36.216	36.293	10	225.4
	34.410				35.972				36.272			
	34.182				36.080				36.391			
12	35.834	35.976	10	223.5	36.878	36.956	10	229.5	39.354	39.027	10	242.4
	36.063				37.016				38.856			
	36.031				36.973				38.872			
13	34.637	34.713	10	215.6	39.294	39.173	10	243.3	38.173	38.248	10	237.6
	34.706				38.945				38.372			
	34.797				39.280				38.199			
14	34.242	34.259	10	212.8	38.398	38.458	10	238.9	35.047	35.016	10	217.5
	34.401				38.349				35.041			
	34.134				38.628				34.959			
15	34.681	34.769	10	216.0	34.802	34.916	10	216.9	37.212	37.332	10	231.9
	34.761				34.819				37.388			
	34.865				35.128				37.397			
17	36.082	36.074	10	224.1	38.488	38.520	10	239.3	41.257	41.353	10	256.8
	36.121				38.548				41.370			
	36.019				38.525				41.431			

[Table 17]

Condition	0 Week		2 Week		4 Week	
	Meas. OD340	Average	Meas. OD340	Average	Meas. OD340	Average
2	0.120	0.119	0.105	0.105	0.140	0.141
	0.117		0.104		0.143	
	0.121		0.106		0.141	
3	0.102	0.101	0.104	0.106	0.126	0.121
	0.101		0.108		0.118	
	0.099		0.106		0.119	
5	0.113	0.106	0.133	0.131	0.161	0.160
	0.102		0.129		0.158	
	0.103		0.131		0.160	
8	0.090	0.090	0.105	0.107	0.120	0.121
	0.093		0.107		0.121	
	0.086		0.108		0.122	
9	0.103	0.103	0.111	0.112	0.140	0.141
	0.103		0.110		0.141	
	0.104		0.114		0.143	
10	0.099	0.098	0.114	0.112	0.132	0.133
	0.097		0.109		0.134	
	0.099		0.112		0.133	
11	0.104	0.102	0.117	0.116	0.136	0.136
	0.101		0.117		0.137	
	0.101		0.114		0.135	
12	0.110	0.104	0.096	0.096	0.099	0.101
	0.102		0.097		0.102	
	0.100		0.095		0.101	
13	0.112	0.110	0.100	0.099	0.098	0.099
	0.110		0.099		0.101	
	0.107		0.097		0.097	
14	0.101	0.098	0.108	0.106	0.143	0.142
	0.098		0.107		0.141	
	0.096		0.104		0.142	
15	0.107	0.105	0.099	0.099	0.117	0.117
	0.105		0.102		0.117	
	0.102		0.097		0.117	
17	0.122	0.119	0.106	0.104	0.114	0.115
	0.118		0.104		0.116	
	0.116		0.103		0.114	

Example 2.2.2. Results of aggregate and fragment analysis

SEC-HPLC was used to compare and evaluate the amount of aggregates and fragments increased for each excipient combination condition (Table 18 and Figure 7). In addition, SEC-HPLC data for a total of 17 conditions, including the excipient only condition test of Example

1.1, was analyzed by ANOVA. As a result, it was confirmed that L-arginine hydrochloride reduced the formation of aggregates at a statistically significant level ($p < 0.01$) (Figure 6), and there was no excipient that reduced the formation of fragments to a statistically significant level.

In addition, as a result of comparing the effect values on aggregation and fragmentation, it was confirmed that the formation of aggregates and fragments of HL161BKN was reduced when L-histidine, D-mannitol, and D-sorbitol were used as excipients. However, there was no synergistic effect between the excipients. D-mannitol and D-sorbitol are isomers, and D-mannitol, which is frequently used among them, was selected (Figure 8).

[Table 18]

Condition No.	Excipients	Relative purity (monomer)			Changes at week 2 (W2-W0)		Changes at week 4 (W4-W0)	
		Week 0	Week 2	Week 4	Δ Aggre	Δ Frag	Δ Aggre	Δ Frag
2	H+S+G	100.0	95.7	95.7	2.3	1.8	1.8	2.2
3	H+A+S	100.0	95.9	96.5	2.1	1.8	1.1	2.2
5	H+A+G	100.0	95.2	95.1	2.3	2.2	2.1	2.5
8	H+A+M	100.0	96.5	97.2	1.4	1.8	0.4	2.3
9	H+M+G	100.0	95.4	95.8	2.5	1.9	1.8	2.2
10	H+A+S+M+G	100.0	95.9	96.2	2.1	1.7	1.3	2.3
11	H+S+M	100.0	95.7	95.7	2.2	1.8	1.9	2.1
12	A+M+G	100.0	96.7	96.3	1.1	2.0	0.9	2.5
13	S+M+G	100.0	96.6	96.6	1.1	2.0	0.7	2.4
14	A+S+G	100.0	97.5	94.7	0.0	2.3	0.6	4.3
15	A+S+M	100.0	96.9	97.2	0.6	2.2	-0.4	3.0
17	(-) Control	100.0	92.6	88.9	4.1	2.7	6.6	3.6

* A: 50 mM L-arginine hydrochloride, H: 50 mM L-histidine, G: 100 mM L-glycine, M: 200 mM D-mannitol, S: 250 mM D-sorbitol

Example 2.2.3. Results of charge variant analysis

The pattern of changes in charge variants of HL161BKN according to the excipient combination condition was confirmed using CEX-HPLC. As a result, no distinct changes in charge variants (basic and acidic variants) could be observed (Figure 9).

Example 2.2.4. Results of viscosity and osmolality analysis

As a result of measuring the viscosity for each excipient combination condition, it was confirmed that the viscosity was reduced when L-arginine hydrochloride was added. The osmolality was increased as the number and concentration of added excipients were increased. Specifically, it was confirmed that the addition of 50 mM L-arginine hydrochloride, 50 mM L-

histidine, and 100 mM L-glycine increased the osmolality of about 100 mOsmol/kg, respectively; the addition of 200 mM D-mannitol increased the osmolality of about 200 mOsmol/kg; and the addition of 250 mM D-sorbitol increased the osmolality of about 250 mOsmol/kg (Table 19).

In general, the osmolality of subcutaneous injections is similar to the osmolality of the body (about 290 mOsmol/kg) and is regulated in the range of about 250 to 500 mOsmol/kg (PCT/EP2009/066675). Therefore, it was determined that the osmolality of the formulation buffer should be regulated in the range of about 220 to 450 mOsmol/kg in consideration of the HL161BKN concentration. Thus, it was decided to proceed with the subsequent test for excipient concentration screening in consideration of the osmolality range.

[Table 19]

Condition No.	Excipients	Viscosity (cP, 25°C)		Osmolality (mOsmol/kg)	
		Buffer	Sample	Buffer	Sample
2	H+S+G	1.066	N/A	498	683
3	H+A+S	1.052	16.700	485	617
5	H+A+G	0.925	19.641	320	395
8	H+A+M	1.002	15.941	434	549
9	H+M+G	1.021	14.807	451	593
10	H+A+S+M+G	1.198	27.865	831	1103
11	H+S+M	1.465	23.680	631	850
12	A+M+G	1.250	39.732	425	560
13	S+M+G	1.055	22.291	471	613
14	A+S+G	1.358	32.547	606	814
15	A+S+M	1.199	19.176	607	788
17	(-) Control	1.115	57.890	23	37

* A: 50 mM L-arginine hydrochloride, H: 50 mM L-histidine, G: 100 mM L-glycine, M: 200 mM D-mannitol, S: 250 mM D-sorbitol

Example 2.3. Results of additional excipient test

An additional excipient test was performed. As a result, 50 mM histidine (pH 6.0) was used as a basal buffer, and L-methionine, which had the effect of inhibiting the formation of aggregates, and 0.02% PSB20, which inhibited the formation of aggregates under the agitation stress conditions, were selected as additional excipients.

Example 2.3.1. Results of L-methionine and histidine basal buffer test

1) Results of concentration (A280) and turbidity (A340) analysis

The HL161BKN concentration was increased for 4 weeks, which was assumed to be due to buffer evaporation under accelerated conditions of 40°C (Table 20). The turbidity was not increased or showed a slight increase to 0.1 or less (Table 21).

[Table 20]

Condition	T0				T2				T4			
	Meas. OD280	Average	Dilution factor	Conc.	Meas. OD280	Average	Dilution factor	Conc.	Meas. OD280	Average	Dilution factor	Conc.
10 mM Na Citrate, pH 6	33.102	33.179	10	206.1	37.997	38.120	10	236.8	34.364	34.382	10	213.6
	33.293				37.989				34.337			
	33.143				38.374				34.446			
10 mM Na Citrate, 50 mM Methionine, pH 6	32.352	32.289	10	200.6	34.964	34.778	10	216.0	33.135	33.026	10	205.1
	32.215				34.658				33.137			
	32.301				34.712				32.805			
10 mM Na Citrate, 50 mM Histidine-HCl, pH 6	35.852	35.817	10	222.5	40.067	40.309	10	250.4	38.534	38.685	10	240.3
	35.773				40.413				38.776			
	35.826				40.447				38.746			
10 mM Na Citrate, 0.02% PSB20, pH 6	33.336	33.529	10	208.3	37.538	37.818	10	234.9	33.866	33.795	10	209.9
	33.572				37.905				33.662			
	33.678				38.012				33.857			
10 mM Histidine, pH 6	32.976	32.987	10	204.9	37.096	37.358	10	232.0	37.282	37.254	10	231.4
	33.024				37.358				37.246			
	32.960				37.619				37.234			
50 mM Histidine, pH 6	33.019	32.937	10	204.6	37.23	37.071	10	230.3	39.888	39.889	10	247.8
	32.837				37.024				40.019			
	32.954				36.959				39.761			

[Table 21]

Condition	T0		T2		T4	
	Meas. OD340	Average	Meas. OD340	Average	Meas. OD340	Average
10 mM Na Citrate, pH 6	0.110	0.111	0.116	0.116	0.127	0.125
	0.115		0.117		0.124	
	0.109		0.114		0.124	
10 mM Na Citrate, 50 mM Methionine, pH 6	0.112	0.112	0.100	0.101	0.103	0.103
	0.115		0.106		0.104	
	0.108		0.098		0.103	
10 mM Na Citrate, 50 mM Histidine, pH 6	0.111	0.108	0.118	0.119	0.135	0.135
	0.107		0.122		0.136	
	0.105		0.117		0.134	
10 mM Na Citrate, 0.02% PSB20, pH 6	0.114	0.115	0.123	0.120	0.123	0.123
	0.115		0.119		0.123	
	0.116		0.117		0.122	
10 mM Histidine, pH 6	0.102	0.103	0.116	0.116	0.149	0.146
	0.108		0.118		0.143	
	0.100		0.115		0.146	
50 mM Histidine, pH 6	0.097	0.095	0.125	0.123	0.153	0.152
	0.096		0.122		0.152	
	0.092		0.123		0.150	

2) Results of aggregate and fragment analysis

Compared with the basal buffer condition, L-methionine exhibited the excellent effect of inhibiting the formation of aggregates. In addition, there was no increase in the formation of aggregates by 0.02% PSB20. In addition, the results of the condition in which L-histidine was added as an excipient was similar to that of the condition in which it was used as a basal buffer, and the formation of aggregates was reduced in the 50 mM histidine condition compared to that in the 10 mM histidine condition (Table 22 and Figure 10).

[Table 22]

Condition No.	Relative purity (monomer)			Changes at week 2 (W2-W0)		Changes at week 4 (W4-W0)	
	Week 0	Week 2	Week 4	Δ Aggre	Δ Frag	Δ Aggre	Δ Frag
1	100.0	90.1	82.5	5.1	3.9	9.9	6.0
2	100.0	95.7	92.4	2.2	1.8	4.3	2.7
3	100.0	95.1	93.9	2.7	1.9	3.0	2.7
4	100.0	88.8	83.5	5.8	4.4	7.7	7.2
5	100.0	94.4	91.9	3.3	1.9	4.4	3.1
6	100.0	96.2	95.1	1.8	1.7	1.8	2.8

3) Results of charge variant analysis

As a result of CEX-HPLC analysis, no distinct changes in charge variants (basic and acidic variants) could be observed (Figure 11).

Example 2.3.2. Results of polysorbate 20 test

1) Results of concentration (A280) and turbidity (A340) analysis

As a result of the agitation test at room temperature, there was little change in concentration. The sample agitated without PSB20 turned white visually and the turbidity could not be measured (Figure 12). On the other hand, the sample with PSB20 was visually slightly cloudy under agitation condition, and the turbidity was increased by about 0.227 (Table 23).

Through this, it was confirmed that PSB20 has an effect of protecting high concentration HL161BKN against the stress caused by agitation.

[Table 23]

PSB20	Agitation	Relative purity (monomer)			Turbidity (A340)		
		Week 0	Week 1	Δ conc. (W1-W0)	Week 0	Week 1	Δ A340 (W1-W0)
-	-	206.1	206.6	0.5	0.111	0.115	0.004
-	+	206.1	NA*	NA	0.111	NA	NA
0.02%	-	208.3	197.4	-10.9	0.115	0.126	0.011
0.02%	+	208.3	212.3	4.0	0.115	0.342	0.227

*NA: Not applicable

2) Results of aggregate and fragment analysis

The formation of aggregates was increased by agitation stress, and it was confirmed that PSB20 had the effect of inhibiting the formation of aggregates. On the other hand, there was no increase in fragments due to agitation stress (Table 24 and Figure 13).

[Table 24]

PSB20	Agitation	Relative purity (monomer)		Week 1 (W1-W0)	
		Week 0	Week 1	Δ Aggre	Δ Frag
-	-	100.0	94.7	4.6	0.2
-	+	100.0	NA	NA	NA-
0.02%	-	100.0	94.3	5.0	0.2
0.02%	+	100.0	90.6	8.3	0.2

*NA: Not applicable

Example 2.3.3. Results of screening of excipient for viscosity reduction

1) Results of concentration (A280) and viscosity analysis

The HL161BKN concentration was increased for 4 weeks (Table 25), which was assumed to be due to buffer evaporation under accelerated conditions of 40°C. When L-histidine hydrochloride, L-arginine hydrochloride, and L-glycine were added, the viscosity was reduced compared to Condition 1, but the effect thereby was at an insignificant. On the other hand, in the case of L-lysine hydrochloride, NaCl, Na₂SO₄, and NH₄Cl, the viscosity was rather greatly increased (Table 26).

[Table 25]

Condition			0 Week				2 Week				4 Week			
			Meas. OD280	Average	Dilution Factor	Conc.	Meas. OD280	Average	Dilution Factor	Conc.	Meas. OD280	Average	Dilution Factor	Conc.
210 mg/mL	1	50 mM Histidine, pH 6	33.850	33.934	10	210.8	38.622	38.502	10	239.1	41.008	41.277	10	256.4
			33.922				38.514				41.264			
			34.031				38.369				41.559			
	2	50 mM Histidine, 100 mM His-HCl, pH 6	32.763	32.539	10	202.1	35.180	35.132	10	218.2	38.764	38.979	10	242.1
			32.386				35.054				39.112			
			32.469				35.161				39.061			
	3	50 mM Histidine, 100 mM Glycine, pH 6	32.403	32.500	10	201.9	36.711	36.544	10	227.0	36.376	36.440	10	226.3
			32.556				36.893				36.527			
			32.541				36.028				36.418			
	4	50 mM Histidine, 100 mM Arg-HCl, pH 6	32.554	32.619	10	202.6	37.297	37.092	10	230.4	36.304	36.403	10	226.1
			32.649				36.950				36.488			
			32.655				37.028				36.418			
	5	50 mM Histidine, 100 mM Lysine-HCl, pH 6	33.940	34.026	10	211.3	37.492	37.491	10	232.9	40.240	40.630	10	252.4
			34.269				37.509				40.916			
			33.870				37.471				40.733			
	6	50 mM Histidine, 100 mM Valine, pH 6	35.426	35.637	10	221.3	38.475	38.518	10	239.2	37.653	37.850	10	235.1
			35.763				38.581				38.070			
			35.722				38.499				37.828			
	7	50 mM Histidine, 100 mM NH ₄ Cl, pH 6	35.017	34.997	10	217.4	37.585	37.575	10	233.4	39.127	39.392	10	244.7
			35.230				37.434				39.621			
			34.743				37.707				39.428			
	8	50 mM Histidine, 100 mM NaCl, pH 6	35.734	35.647	10	221.4	37.912	37.844	10	235.1	38.064	38.115	10	236.7
			35.597				37.807				38.364			
			35.611				37.812				37.917			
	9	50 mM Histidine, 100 mM Na ₂ SO ₄ , pH 6	35.553	35.655	10	221.5	35.367	35.383	10	219.8	36.852	37.118	10	230.5
			35.792				35.440				37.310			
			35.620				35.343				37.191			

[Table 26]

Condition No.	Excipients	Viscosity (cP, 25°C)	
		Buffer	Sample
1	-	1.047	19.723
2	L-His HCl	1.240	18.487
3	L-Lys HCl	1.101	26.472
4	L-Arg HCl	1.105	17.768
5	L-Glycine	1.070	17.716
6	L-Valine	1.086	20.753
7	NaCl	0.980	25.620
8	Na ₂ SO ₄	1.088	24.135
9	NH ₄ Cl	1.049	30.414

2) Results of aggregate and fragment analysis

The aggregates and fragments were analyzed by SEC-HPLC analysis. As a result, when L-histidine hydrochloride and L-arginine hydrochloride were added, relatively few aggregates were formed compared to Condition 1, and there was no excipient to reduce the formation of fragments (Table 27 and Figure 14).

[Table 27]

Condition No.	Excipients	Relative purity (monomer%)			Changes at week 2 (W2-W0)		Changes at week 4 (W4-W0)	
		Week 0	Week 2	Week 4	ΔAggre	ΔFrag	ΔAggre	ΔFrag
1	-	100.0	92.4	89.4	5.2	2.2	6.9	3.3
2	L-His HCl	100.0	94.4	92.4	3.0	2.4	3.9	3.5
3	L-Lys HCl	100.0	93.7	92.1	3.8	2.3	4.5	3.1
4	L-Arg HCl	100.0	94.6	92.8	3.0	2.2	3.8	3.2
5	L-Glycine	100.0	93.7	91.7	3.9	2.2	4.9	3.2
6	L-Valine	100.0	93.6	92.2	3.9	2.3	4.4	3.1
7	NaCl	100.0	93.2	91.4	4.2	2.4	5.3	3.1
8	Na ₂ SO ₄	100.0	94.1	92.3	3.6	2.2	4.4	3.0
9	NH ₄ Cl	100.0	93.4	91.6	4.1	2.3	4.8	3.3

Example 2.4. Results of excipient concentration screening

Example 2.4.1. Results of first excipient concentration screening

In order to screen the concentration of the histidine basal buffer (pH 6.0) selected in Example 1.3 above and three excipients (L-methionine, L-arginine hydrochloride, and D-mannitol) in 0.02% PSB20, a test was performed under a total of 9 conditions (Table 28). As a

result, L-arginine hydrochloride and PSB20 were selected as excipients in addition to the histidine basal buffer.

[Table 28]

Condition No.	L-Met (mM)	L-Arg HCl (mM)	D-Mannitol (mM)	PSB20 (%)
1	-	-	-	-
2	50	50	0	0.02
3	10	100	0	0.02
4	10	50	100	0.02
5	10	100	100	0.02
6	50	100	100	0.02
7	50	50	100	0.02
8	50	100	0	0.02
9	10	50	0	0.02

1) Results of concentration (A280) and turbidity (A340) analysis

The HL161BKN concentration was increased for 4 weeks by about 15%, which was assumed to be due to buffer evaporation under accelerated conditions of 40°C (Table 29). The turbidity in each condition showed only an insignificant increase of about 0.048 on average (Table 30).

[Table 29]

Condition			0 Week				2 Week				4 Week			
			Meas. OD280	Average	Dilution Factor	Conc.	Meas. OD280	Average	Dilution Factor	Conc.	Meas. OD280	Average	Dilution Factor	Conc.
210 mg/mL	1	50 mM Histidine, pH 6	34.818	34.673	10	215.4	40.515	40.787	10	253.3	39.391	39.579	10	245.8
			34.770				40.963				39.619			
			34.432				40.883				39.726			
	2	50 mM Histidine, 50 mM Methionine, 50 mM Arg-HCl, pH 6	35.636	35.605	10	221.1	41.353	41.430	10	257.3	40.936	41.245	10	256.2
			35.538				41.565				41.451			
			35.641				41.372				41.349			
	3	50 mM Histidine, 10 mM Methionine, 100 mM Arg-HCl, pH 6	33.558	33.430	10	207.6	36.078	36.064	10	224.0	34.138	34.161	10	212.2
			33.443				36.145				34.256			
			33.289				35.968				34.088			
	4	50 mM Histidine, 10 mM Methionine, 50 mM Arg-HCl, 100 mM Mannitol, pH 6	36.486	36.523	10	226.9	40.730	40.791	10	253.4	40.325	40.219	10	249.8
			36.690				40.785				40.367			
			36.393				40.857				39.965			
	5	50 mM Histidine, 10 mM Methionine, 100 mM Arg-HCl, 100 mM Mannitol, pH 6	36.410	36.336	10	225.7	36.240	36.254	10	225.2	37.971	38.208	10	237.3
			36.131				36.256				38.410			
			36.467				36.266				38.243			
	6	50 mM Histidine, 50 mM Methionine, 100 mM Arg-HCl, 100 mM Mannitol, pH 6	35.108	35.125	10	218.2	39.423	39.641	10	246.2	41.750	41.923	10	260.4
			35.132				39.749				41.992			
			35.136				39.752				42.027			
	7	50 mM Histidine, 50 mM Methionine, 50 mM Arg-HCl, 100 mM Mannitol, pH 6	36.423	36.579	10	227.2	39.168	39.110	10	242.9	43.308	43.031	10	267.3
			36.555				39.018				42.800			
			36.759				39.144				42.985			
	8	50 mM Histidine, 50 mM Methionine, 100 mM Arg-HCl, pH 6	34.828	35.081	10	217.9	35.673	35.929	10	223.2	37.477	37.722	10	234.3
			35.124				35.967				37.909			
			35.292				36.146				37.779			
	9	50 mM Histidine, 10 mM Methionine, 50 mM Arg-HCl, pH 6	31.656	31.625	10	196.4	35.713	35.849	10	222.7	34.000	34.103	10	211.8
			31.604				35.884				34.226			
			31.615				35.951				34.084			

[Table 30]

Condition	T0		T2		T4	
	Meas. OD340	Average	Meas. OD340	Average	Meas. OD340	Average
50 mM Histidine, pH 6	0.148	0.147	0.170	0.170	0.189	0.191
	0.146		0.169		0.191	
	0.147		0.170		0.192	
50 mM Histidine, 50 mM Methionine, 50 mM Arg-HCl, pH 6	0.145	0.143	0.186	0.182	0.194	0.193
	0.143		0.183		0.191	
	0.141		0.178		0.193	
50 mM Histidine, 10 mM Methionine, 100 mM Arg-HCl, pH 6	0.124	0.123	0.167	0.164	0.180	0.178
	0.122		0.161		0.178	
	0.123		0.165		0.175	
50 mM Histidine, 10 mM Methionine, 50 mM Arg-HCl, 100 mM Mannitol, pH 6	0.149	0.147	0.183	0.181	0.202	0.199
	0.148		0.179		0.198	
	0.145		0.182		0.197	
50 mM Histidine, 10 mM Methionine, 100 mM Arg-HCl, 100 mM Mannitol, pH 6	0.134	0.134	0.173	0.172	0.185	0.184
	0.132		0.172		0.184	
	0.135		0.172		0.184	
50 mM Histidine, 50 mM Methionine, 100 mM Arg-HCl, 100 mM Mannitol, pH 6	0.132	0.133	0.172	0.172	0.191	0.190
	0.133		0.170		0.191	
	0.134		0.174		0.188	
50 mM Histidine, 50 mM Methionine, 50 mM Arg-HCl, 100 mM Mannitol, pH 6	0.159	0.160	0.180	0.178	0.192	0.194
	0.160		0.175		0.196	
	0.162		0.180		0.193	
50 mM Histidine, 50 mM Methionine, 100 mM Arg-HCl, pH 6	0.139	0.138	0.175	0.174	0.191	0.189
	0.139		0.173		0.188	
	0.137		0.174		0.189	
50 mM Histidine, 10 mM Methionine, 50 mM Arg-HCl, pH 6	0.141	0.142	0.164	0.163	0.186	0.184
	0.143		0.160		0.184	
	0.141		0.164		0.182	

2) Results of aggregate and fragment analysis

As a result of SEC-HPLC analysis, the amount of aggregates and fragments increased according to each excipient concentration condition did not show a significant difference. However, the stability in the basal buffer condition (Condition 1) of 50 mM histidine was high, and the formation of aggregates was reduced when 100 mM L-arginine hydrochloride was added rather than 50 mM. In addition, it was confirmed that the amount of aggregates and fragments increased according to the concentration of L-methionine and the presence or absence of D-mannitol added had little difference or a slight increase from the basal buffer condition (Table 31 and Figure 15).

[Table 31]

Condition No.	Relative purity (monomer%)			Changes at week 2 (W2-W0)		Changes at week 4 (W4-W0)	
	Week 0	Week 2	Week 4	Δ Aggre	Δ Frag	Δ Aggre	Δ Frag
1	100.0	96.5	94.8	1.7	1.6	2.6	2.4
2	100.0	95.8	94.2	2.3	1.7	2.9	2.6
3	100.0	96.5	94.6	1.7	1.6	2.6	2.6
4	100.0	95.6	94.1	2.6	1.6	3.3	2.3
5	100.0	96.1	94.7	2.0	1.8	2.6	2.5
6	100.0	95.9	94.3	2.2	1.7	2.8	2.6
7	100.0	95.9	94.1	2.2	1.7	3.2	2.5
8	100.0	95.9	94.5	2.2	1.7	2.8	2.5
9	100.0	94.9	93.0	2.6	2.4	3.4	3.3

3) Results of charge variant analysis

As a result of CEX-HPLC analysis, no distinct changes in charge variants were observed according to each excipient condition (Figure 16).

4) Results of viscosity and osmolality analysis

As a result of measuring the viscosity and osmolality according to each excipient concentration condition, it was confirmed that the effect of reducing the viscosity was high in the condition in which L-arginine hydrochloride was added at a concentration of 100 mM rather than 50 mM, and most of them had 112 to 588 mOsmol/kg to satisfy the osmolality criteria (Table 32).

[Table 32]

Condition No.	Viscosity(cP, 25°C)				Osmolality (mOsmol/kg)	
	Week 0		Week 4		Week 0	
	Buffer	Sample	Sample	Δ viscosity (W4-W0)	Buffer	Sample
1	1.062	18.682	25.033	6.321	80	112
2	1.081	16.426	28.315	11.889	232	297
3	1.120	12.148	15.029	2.881	283	331
4	1.126	19.706	30.707	11.001	305	408
5	1.176	17.528	18.147	0.619	401	500
6	1.185	17.800	29.186	11.386	446	588
7	1.168	25.624	28.682	3.058	349	474
8	1.133	18.321	18.282	-0.039	335	432
9	1.081	13.958	14.756	0.798	192	238

Example 2.4.2. Second excipient concentration screening

Among the histidine basal buffer selected in the first excipient concentration screening of Example 1.4.1 and two excipients, in order to select the optimal concentration of L-arginine hydrochloride, 2 batch samples of HL161BKN (HL161BKN B018 and HL161BKN B021) were evaluated for the stability through the concentration, turbidity, purity (aggregates and fragments), viscosity, osmolality test, and suitability for subcutaneous injection (SC) administration in four conditions. As a result, 100 mM L-histidine, 100 mM L-arginine hydrochloride, 0.02% PSB20, pH 6.0 was determined as the final formulation of HL161BKN (Table 33).

[Table 33]

Conditions	L-Histidine (Basal buffer) (mM)	L-Arg-HCl (mM)	PSB20 (%)	HL161BKN Batch#
1	50	100	0.02	B018
2	50	150	0.02	
3	100	100	0.02	
4	150	50	0.02	
1	50	100	0.02	B021
2	50	150	0.02	
3	100	100	0.02	
4	150	50	0.02	

1) Concentration (A280) and turbidity (A340) analysis

There was little change in the HL161BKN concentration for 4 weeks, which was expected to be an effect of preventing evaporation of the buffer by sealing the 1.5 mL microcentrifuge with parafilm and then storing it in a thermo-hygrostat (Table 34). In addition, the turbidity under all conditions showed an insignificant increase of 0.102 or less (Table 35).

[Table 34]

Condition			0 Week				2 Week				4 Week			
			Meas. OD280	Average	Dilution Factor	Conc.	Meas. OD280	Average	Dilution Factor	Conc.	Meas. OD280	Average	Dilution Factor	Conc.
B018 (pool)	1	50 mM Histidine, 100 mM Arg-HCl, pH 6	35.485	35.547	10	220.8	34.738	34.727	10	215.7	34.493	34.474	10	214.1
			35.354				34.556				34.600			
			35.801				34.887				34.329			
	2	50 mM Histidine, 150 mM Arg-HCl, pH 6	36.112	36.128	10	224.4	36.017	36.022	10	223.7	34.073	34.095	10	211.8
			36.154				36.057				34.010			
			36.117				35.992				34.201			
	3	100 mM Histidine, 100 mM Arg-HCl, pH 6	37.151	37.273	10	231.5	35.054	35.005	10	217.4	33.163	33.265	10	206.6
			37.272				35.016				33.197			
			37.396				34.946				33.434			
	4	150 mM Histidine, 50 mM Arg-HCl, pH 6	35.793	35.843	10	222.6	33.970	33.994	10	211.1	33.999	34.049	10	211.5
			35.722				34.009				34.146			
			36.015				34.003				34.003			
B021 (13D7)	1	50 mM Histidine, 100 mM Arg-HCl, pH 6	34.958	35.085	10	217.9	36.516	36.453	10	226.4	35.538	35.633	10	221.3
			35.292				36.348				35.522			
			35.004				36.494				35.838			
	2	50 mM Histidine, 150 mM Arg-HCl, pH 6	35.187	35.346	10	219.5	32.950	32.886	10	204.3	35.910	35.973	10	223.4
			35.504				32.955				35.990			
			35.346				32.753				36.019			
	3	100 mM Histidine, 100 mM Arg-HCl, pH 6	33.123	33.218	10	206.3	36.664	36.632	10	227.5	34.242	34.278	10	212.9
			33.329				36.712				34.408			
			33.203				36.520				34.184			
	4	150 mM Histidine, 50 mM Arg-HCl, pH 6	35.554	35.584	10	221.0	38.367	38.305	10	237.9	36.008	36.052	10	223.9
			35.600				38.500				36.075			
			35.597				38.048				36.072			

[Table 35]

Condition			T0		T2		T4		Δ OD340 (T4-T0)
			Meas. OD340	Average	Meas. OD340	Average	Meas. OD340	Average	
B018 (pool)	1	50 mM Histidine, 100 mM Arg-HCl, pH 6	0.068	0.066	0.084	0.082	0.094	0.092	0.026
			0.068		0.080		0.092		
			0.063		0.083		0.091		
	2	50 mM Histidine, 150 mM Arg-HCl, pH 6	0.060	0.060	0.084	0.082	0.092	0.091	0.031
			0.063		0.080		0.092		
			0.057		0.083		0.090		
	3	100 mM Histidine, 100 mM Arg-HCl, pH 6	0.065	0.064	0.084	0.082	0.100	0.099	0.034
			0.065		0.082		0.100		
			0.063		0.080		0.096		
	4	150 mM Histidine, 50 mM Arg-HCl, pH 6	0.064	0.061	0.084	0.083	0.107	0.107	0.046
			0.060		0.082		0.109		
			0.060		0.082		0.105		
Life tech (13D7)	1	50 mM Histidine, 100 mM Arg-HCl, pH 6	0.140	0.142	0.214	0.216	0.244	0.244	0.102
			0.143		0.218		0.245		
			0.142		0.216		0.242		
	2	50 mM Histidine, 150 mM Arg-HCl, pH 6	0.139	0.140	0.187	0.186	0.202	0.201	0.061
			0.139		0.186		0.200		
			0.142		0.186		0.202		
	3	100 mM Histidine, 100 mM Arg-HCl, pH 6	0.135	0.138	0.195	0.193	0.213	0.214	0.076
			0.142		0.193		0.215		
			0.136		0.192		0.213		
	4	150 mM Histidine, 50 mM Arg-HCl, pH 6	0.144	0.140	0.203	0.203	0.230	0.230	0.090
			0.137		0.205		0.229		
			0.139		0.201		0.231		

2) Aggregate and fragment analysis

Through SEC-HPLC analysis, the amount of aggregates and fragments increased according to each excipient condition did not show a significant difference, but it was confirmed that the formation of aggregates was the least in Condition 3 (100 mM L-Histidine, 100 mM L-Arginine HCl, 0.02% PSB20, pH 6.0) and Condition 4 (50 mM L-Histidine, 100 mM L-Arginine HCl, 0.02% PSB20, pH 6.0) (Table 36 and Figure 17).

[Table 36]

Batch No.	Condition No.	Relative purity (monomer)			Changes at week 2 (W2-W0)		Changes at week 4 (W4-W0)	
		Week 0	Week 2	Week 4	Δ Aggre	Δ Frag	Δ Aggre	Δ Frag
B018	1	100.0	96.6	94.9	1.5	1.9	2.3	2.7
	2	100.0	96.3	95.2	1.7	2.0	2.2	2.6
	3	100.0	96.7	95.3	1.3	2.0	1.8	2.8
	4	100.0	96.7	95.5	1.1	2.1	1.6	2.9
B021	1	100.0	96.1	94.1	1.5	2.3	2.1	3.7
	2	100.0	96.0	94.3	1.7	2.2	2.2	3.4
	3	100.0	96.4	94.6	1.3	2.2	1.6	3.7
	4	100.0	96.3	94.7	1.3	2.4	1.6	3.7

3) Charge variant analysis

As a result of CEX-HPLC analysis, no distinct changes in charge variants (basic and acidic variants) was observed according to each excipient condition (Figure 18).

4) Viscosity and osmolality analysis

As a result of measuring the viscosity and osmolality according to each excipient concentration condition, the viscosity was regulated to 11 to 16 cP, i.e., below the 20 cp of limit, which is the viscosity limit of the subcutaneous administration product, and the osmolality was 350 to 465 mOsmol/kg to satisfy the criteria of 250 to 500 mOsmol/kg (Table 37).

[Table 37]

Batch No.	Condition No.	Viscosity (cP, 25°C)			Osmolality (mOsmol/kg)			
		Week 0		Week 4	Week 0		Week 4	
		Buffer	Sample	Sample	Buffer	Sample	Sample	Δ mOsm (W4-W0)
B018	1	1.146	10.956	14.067	271	350	360	10
	2	1.160	13.126	12.117	360	429	436	7
	3	1.162	13.548	12.024	367	443	446	3
	4	1.153	11.848	11.777	373	465	475	10
B021	1	1.146	13.466	13.834	271	355	434	79
	2	1.160	14.030	11.871	360	453	458	5
	3	1.162	13.132	11.881	367	448	453	5
	4	1.153	15.875	13.351	373	465	471	6

5) Analysis of insoluble sub-visible particles

Analysis of insoluble sub-visible particles for each excipient condition sample of HL161BKN using micro flow imaging (MFI) was conducted at New Drug Development Support

Center of Osong Hi-tech Medical Industry Promotion Foundation.

Samples of each condition were diluted to 10 mg/mL to compare the increase in the number of sub-visible particles in the range of 5 μm to 100 μm . As a result, the increase in the number of sub-visible particles was the least in Condition 3 (Table 38 and Figure 19).

[Table 38]

Condition No.	Δ Sub-visible particle number (W4-W1)					Total Sub-visible particle number (5 ~ 100 μm)		
	$5 \leq x < 10 \mu\text{m}$	$10 \leq x < 25 \mu\text{m}$	$25 \leq x < 50 \mu\text{m}$	$50 \leq x < 75 \mu\text{m}$	$75 \leq x < 100 \mu\text{m}$	Week 1	Week 4	Δ Particle (W4-W1)
1	30768	8779	402	26	5	2296	42131	39835
2	14463	3059	240	15	4	949	18920	17971
3	5680	1787	163	16	3	966	8516	7550
4	5940	1658	134	7	1	703	8586	7883

Example 3. Stability test

A 36-month long-term storage and accelerated stability test under the final selected formulation conditions for HL161BKN was performed by Catalent (USA). In order to evaluate the stability, the drug product (DP) was stored using a borosilicate glass vial and a teflon-coated rubber stopper. As a result of confirming the stability under the selected formulation conditions, the stability of DP was confirmed for 36 months, thereby securing the possibility of development as an injection (Table 39 and Table 40).

[Table 39]

Drug product, long term stability at 5 ± 3°C										
Test item	Specification	Test Timepoints (Month)								
		0	1	3	6	9	12	18	24	36
Appearance	Clear, colorless to slightly yellow liquid in a clear, colorless glass vial; essentially free of particles	Clear slightly yellow liquid in a clear, colorless glass vial. Essentially free of particles	Clear slight yellow liquid in a clear, colorless glass vial, free of particles	Clear slightly yellow liquid in a clear, colorless glass vial; essentially free from particles	Clear slightly yellow liquid in a clear, colorless glass vial; essentially free of particles	Clear slightly yellow liquid in a clear, colorless glass vial; essentially free of particles	Clear slightly yellow liquid in a clear, colorless glass vial; essentially free of particles	Clear slightly yellow liquid in a clear, colorless glass vial; essentially free of particles	Clear slightly yellow liquid in a clear, colorless glass vial; essentially free of particles	Clear slightly yellow liquid in a clear, colorless glass vial; essentially free of particles
pH	5.5 - 6.5	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
A280	153 - 187 mg/mL	176 mg/mL	169 mg/mL	174 mg/mL	175 mg/mL	177 mg/mL	174 mg/mL	174 mg/mL	179 mg/mL	176 mg/mL
CE-SDS, Reduced	Sum of Heavy Chain and Light Chain ≥ 95.0%	98.6%	98.4%	98.4%	98.5%	98.1%	97.9%	97.8%	98.0%	97.7%
CE-SDS, Non-Reduced ¹⁾	Report Intact Antibody (XX.X%)	89.9%	96.0%	95.8%	96.0%	95.4%	96.0%	95.8%	94.5%	95.1%
SEC-HPLC	≥ 90.0% Main Peak Aggregates and fragment ≤ 10.0%	99.4% <LLOQ	99.6% <LLOQ	98.4% 1.6%	98.8% 1.2%	99.4% <LLOQ	98.8% 1.2%	96.4% 3.6%	98.5% 1.5%	98.5% 1.5%
cIEF	Report Main peak pI value Report % Area of Basic group Report % Area of Main group Report % Area of Acidic group	pI=7.75 B: 1.25% M: 75.61% A: 23.14%	pI=7.78 B: 2.01% M: 77.17% A: 20.81%	pI=7.82 B: 2.01% M: 76.78% A: 21.21%	pI=7.80 B: 2.27% M: 75.29% A: 22.44%	pI=7.87 B: 2.12% M: 75.18% A: 22.70%	pI=7.74 B: 0.91% M: 78.55% A: 20.55%	pI=7.85 B: 2.66% M: 72.64% A: 24.70%	pI=7.82 B: 2.67% M: 72.06% A: 25.27%	pI=7.79 B: 2.21% M: 67.72% A: 30.06%
Potency	70 ~ 130% to reference standard	82%	108%	92%	107%	118%	118%	110%	96%	106%
Particulate Matter	≥10 µm: ≤ 6000 particles/container ≥25 µm: ≤ 600 particles/container	≥10 µm: 105 ≥25 µm: 0	Not Tested	≥10 µm: 4356 ≥25 µm: 73	≥10 µm: 14 ≥25 µm: 0	≥10 µm: 17 ≥25 µm: 2	≥10 µm: 42 ≥25 µm: 1	≥10 µm: 55 ≥25 µm: 1	≥10 µm: 143 ≥25 µm: 5	≥10 µm: 373 ≥25 µm: 10
Volume in Container	NLT the labeled volume	1 mL	Not Tested	Not Tested	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
Endotoxin	≤ 0.5 EU/mg of protein	<0.1 EU/mg	Not Tested	Not Tested	Not Tested	Not Tested	<0.1 EU/mg	Not Tested	<0.1 EU/mg	<0.1 EU/mg
Sterility	No growth	No growth	Not Tested	Not Tested	Not Tested	Not Tested	No growth	Not Tested	No growth	No growth

[Table 40]

Drug Product, Accelerated stability at 25 ± 2°C / 60 ± 5%RH					
Test item	Specification	Test Timepoints (Month)			
		0	1	3	6
Appearance	Clear, colorless to slightly yellow liquid in a clear, colorless glass vial, essentially free of particles	Clear slightly yellow liquid in a clear colorless glass vial, essentially free of particles	Clear light yellow liquid in a clear colorless glass vial, essentially free of particles	Clear slightly yellow liquid in a clear, colorless glass vial, essentially free from particles	Clear slightly yellow liquid in a clear, colorless glass vial, essentially free of particles
pH	5.5 - 6.5	5.9	5.9	5.9	5.9
A280	153 - 187 mg/mL	176 mg/mL	169 mg/mL	171 mg/mL	177 mg/mL
CE-SDS, Reduced	Sum of Heavy Chain and Light Chain ≥ 95.0%	98.6%	98.3%	97.9%	97.3%
CE-SDS, Non-Reduced ⁽¹⁾	Report Intact Antibody (XX.X%)	89.9%	95.7%	95.0%	93.7%
SEC-HPLC	≥ 90.0% Main Peak	99.4%	99.1%	97.5%	94.8%
	Aggregates and fragment ≤ 10.0%	<LLOQ ⁽²⁾	0.9%	2.6%	5.2%
cIEF	Report Main peak pI value	pI=7.75	pI=7.81	pI=7.82	pI=7.77
	Report % Area of Basic group	B: 1.25%	B: 2.54%	B: 3.28%	B: 3.98%
	Report % Area of Main group	M: 75.61%	M: 73.72%	M: 67.30%	M: 60.60%
	Report % Area of Acidic group	A: 23.14%	A: 23.74%	A: 29.42%	A: 35.42%
Potency	70 ~ 130% to reference standard	82%	104%	79%	72%
Particulate Matter	≥10 µm: ≤ 6000 particles/container	≥10 µm: 105	Not Tested	≥10 µm: 4478	≥10 µm: 12
	≥25 µm: ≤ 600 particles/container	≥25 µm: 0		≥25 µm: 14	≥25 µm: 0

Example 4. Selection of final development candidate antibody formulation

Since HL161BKN exhibits a tendency to be very stable at a high concentration in the formulation, it was intended to confirm the possibility of development in the form of an injection for subcutaneous administration. When developing an injection, it is known that a viscosity of 20 cP or less is suitable for subcutaneous administration in order to reduce pain and side effects at the site of administration. Accordingly, HL161BKN was concentrated under 9-step concentration conditions, and the viscosity was measured under 5°C and 25°C conditions. As a result of confirming the viscosity of the HL161BKN sample having a high concentration of 170 mg/mL, it was confirmed that it had a viscosity of 10 cP at 25°C, and thus subcutaneous administration was possible (Figure 20).

It was confirmed that HL161BKN had very stable properties even at a high concentration of 200 mg/mL or more in the above formulation study. As a result, it is expected that it will be possible to develop a self-administered SC injection product in the future. Considering that all products of other competing companies are infusion type products, it is possible to be differentiated by increasing patient convenience.

In addition, since it was confirmed that a high concentration of HL161BKN was stable

in the formulation, a low concentration of HL161BKN was also expected to be stable in the formulation. Therefore, the formulation can be applied to HL161BKN at various concentrations.

Claims

[Claim 1]

A pharmaceutical formulation having a pH of 4.0 to 8.0, comprising

- (a) an anti-FcRn antibody or a fragment thereof,
- (b) at least one additive selected from mannitol, sorbitol, arginine, histidine, glycine and salts thereof,
- (c) a buffer system selected from citrate or histidine, and
- (d) a surfactant.

[Claim 2]

The pharmaceutical formulation according to claim 1, wherein the additive is arginine or a salt thereof, and the buffer system is histidine.

[Claim 3]

The pharmaceutical formulation according to claim 1, wherein the surfactant is polysorbate.

[Claim 4]

The pharmaceutical formulation according to claim 1, further comprising methionine.

[Claim 5]

The pharmaceutical formulation according to claim 1, wherein the pharmaceutical formulation is in the form of an injection.

[Claim 6]

The pharmaceutical formulation according to claim 1, wherein the pharmaceutical formulation has a viscosity of 20 cP or less.

[Claim 7]

The pharmaceutical formulation according to claim 1, wherein the pharmaceutical

formulation has an osmolality of 250 mOs/kg to 500 mOs/kg.

[Claim 8]

The pharmaceutical formulation according to claim 1, wherein the concentration of the anti-FcRn antibody or fragment thereof is 50 mg/mL to 250 mg/mL.

[Claim 9]

The pharmaceutical formulation according to claim 8, wherein the concentration of the anti-FcRn antibody or fragment thereof is 80 mg/mL to 250 mg/mL.

[Claim 10]

The pharmaceutical formulation according to claim 1, wherein the pH of the pharmaceutical formulation is 4.0 to 7.0.

[Claim 11]

The pharmaceutical formulation according to claim 1, wherein the anti-FcRn antibody comprises

a heavy chain variable region comprising H-CDR1 having the amino acid of SEQ ID NO: 5, H-CDR2 having the amino acid of SEQ ID NO: 6, and H-CDR3 having the amino acid of SEQ ID NO: 7; and

a light chain variable region comprising L-CDR1 having the amino acid of SEQ ID NO: 8, L-CDR2 having the amino acid of SEQ ID NO: 9, and L-CDR3 having the amino acid of SEQ ID NO: 10.

[Claim 12]

The pharmaceutical formulation according to claim 1, wherein the pharmaceutical formulation is administered subcutaneously.

[Claim 13]

The pharmaceutical formulation according to claim 1, wherein the pharmaceutical formulation comprises

- (a) 50 mg/mL to 250 mg/mL of an anti-FcRn antibody,
 - (b) 50 to 250 mM L-arginine or a hydrochloride salt thereof,
 - (c) 50 to 250 mM L-histidine buffer, and
 - (d) 0.01 to 0.05% polysorbate 20, and
- the pharmaceutical formulation has a pH of 4.0 to 7.0.

[Claim 14]

The pharmaceutical formulation according to claim 1, wherein the pharmaceutical formulation has increased stability, wherein the amount of aggregates and fragments of HL161BKN is 10% or less when stored for 6 months under an accelerated condition (25°C, a relative humidity of 60%).

[Claim 15]

The pharmaceutical formulation according to claim 1, wherein the pharmaceutical formulation has increased stability, wherein the amount of aggregates and fragments of HL161BKN is 10% or less when stored for 36 months under a long-term storage condition (5°C).

[Claim 16]

The pharmaceutical formulation according to claim 1, wherein the pharmaceutical formulation has increased stability, wherein the amount of aggregates and fragments of HL161BKN is 5.0% or less when stored for 36 months under a long-term storage condition (5°C).

[Claim 17]

The pharmaceutical formulation according to any one of claims 1 to 16, wherein the pharmaceutical formulation is for the treatment of an autoimmune disease selected from the group consisting of myasthenia gravis, thyroid eye disease, warm autoimmune hemolytic anemia, neuromyelitis optica, immune thrombocytopenic purpura, pemphigus vulgaris, chronic inflammatory demyelinating polyneuropathy, lupus nephritis, and membranous nephropathy.