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略語及び用語の定義

略語	略していない表現（英語）	略していない表現（日本語）
ACE	affinity capture elution	－
ADA	antidrug antibody(ies)	抗薬物抗体
BEAD	biotin-drug extraction with acid dissociation	ビオチン－薬剤抽出及び酸解離
CIDP	chronic inflammatory demyelinating polyneuropathy	慢性炎症性脱髄性多発根神経炎
CTD	common technical document	コモン・テクニカル・ドキュメント
DDS	Drug Development Solutions	－
ECLIA	electrochemiluminescence immunoassay	電気化学発光免疫測定法
エフガルチギモド	Efgartigimod Alfa (Genetical Recombination)	エフガルチギモド　アルファ（遺伝子組換え）
エフガルチギモド IV	efgartigimod formulation for intravenous infusion	エフガルチギモド静脈内投与用注射製剤
エフガルチギモド PH20 SC	efgartigimod formulation for subcutaneous administration coformulated with rHuPH20	エフガルチギモド　アルファ（遺伝子組換え）にボルヒアルロニダーゼ　アルファ（遺伝子組換え）を配合した皮下投与用注射製剤
ELISA	enzyme-linked immunosorbent assay	酵素結合免疫吸着法
EU	European Union	欧州連合
Fc	crystallizable fragment	－
FcRn	neonatal crystallizable fragment receptor	胎児性 Fc 受容体
gMG	generalized myasthenia gravis	全身型重症筋無力症
IgG	immunoglobulin γ	免疫グロブリン G
ISI	Integrated Summary of Immunogenicity	－
IV	intravenous(ly)	静脈内
IVIg	intravenous immunoglobulin	経静脈的免疫グロブリン療法
IVD	in vitro diagnostic	体外診断用医薬品
NAb	neutralizing antibody(ies)	中和抗体
rHuPH20	recombinant human hyaluronidase PH20	ボルヒアルロニダーゼ　アルファ（遺伝子組換え）
SC	subcutaneous(ly)	皮下

2.7.1　生物薬剤学及び関連する分析法

2.7.1.1　背景及び概観

本申請の対象は、慢性炎症性脱髄性多発根神経炎（CIDP）を適応とする、エフガルチギモド　アルファ（遺伝子組換え）（以下、「エフガルチギモド」）にボルヒアルロニダーゼアルファ（遺伝子組換え）（以下、rHuPH20）を配合した皮下投与用注射製剤である。本配合剤をエフガルチギモド PH20 SC と呼ぶ。

エフガルチギモド（ARGX-113）は、胎児性 Fc 受容体（FcRn）の天然リガンドであるヒト免疫グロブリン G1（IgG1）の Fc フラグメントである。エフガルチギモドは、生理的 pH 下及び酸性 pH 下のいずれでも FcRn との親和性を増大させるように設計されており、内因性 IgG との結合を拮抗阻害し、FcRn を介した IgG のリサイクルを妨げることで IgG 分解を促進させる。エフガルチギモドは、病原性 IgG 自己抗体が介在する CIDP などの重度の自己免疫疾患に対する治療薬として評価されている。エフガルチギモドは、FcRn をブロックすることにより、循環血中の病原性 IgG 自己抗体を減少させる（Vaccaro, 2005; Ulrichts, 2018）。

CIDP は、末梢神経の脱髄による筋力低下と感覚障害を特徴とする、重度な消耗性進行性の末梢神経系の自己免疫疾患である（Bunschoten, 2019a）。CIDP の現行の治療選択肢には、経静脈的免疫グロブリン療法（IVIg）や副腎皮質ステロイドなどがあるが（Van den Bergh, 2010; Van den Bergh, 2021）、CIDP 患者の多くは消耗性の症状に苦しみ、疾患の変動と大きな治療負担を抱え、慢性的な治療が必要になることが多い（Van den Bergh, 2021; Bunschoten, 2019b）。現行の治療選択肢は、長時間の注入、血栓症及び急性腎機能障害などの重度の合併症、並びに体重増加及び骨粗鬆症などの他の望ましくない作用を伴うために長期投与には限界がある（Bunschoten, 2019a; Querol, 2021; Gorson, 2012; Nobile-Orazio, 2012）。

本疾患で IgG 自己抗体が重要な役割を果たしていることを示唆するエビデンスは増えつつある（Querol, 2017; Zinman, 2005; Hahn, 1996; Yan, 2000）。エフガルチギモド PH20 SC は、IgG 自己抗体を含む総 IgG を選択的に減少させる（Ulrichts, 2018）。また、忍容性が高く、良好な安全性プロファイルを有するため、未治療の CIDP 患者及び治療歴のある CIDP 患者に対して、投与方法が簡便で有効な治療選択肢を提供できる可能性がある。

エフガルチギモドの静脈内投与用注射製剤（以下、「エフガルチギモド IV」）は、米国、欧州連合（EU）、日本を含む様々な地域で、全身型重症筋無力症（gMG）を適応とした承認を取得している。さらに、エフガルチギモド PH20 SC は、米国（販売名：Vyvgart Hytrulo）、EU（販売名：Vyvgart）及び日本（販売名：ヒフデュラ配合皮下注）で gMG 患者を対象とした承認を取得しており、様々な地域で審査中である。

CIDP を適応とする本申請に関連するエフガルチギモド IV 及びエフガルチギモド PH20 SC のデータは、提出及び承認済みの gMG を適応とする申請資料概要（以降、「gMG IV CTD」及び「gMG SC CTD」）をそれぞれ引用する。日本の詳細な情報については、モジュール 1 を参照のこと。

2.7.1.1.1　CIDP の適応を裏づける臨床試験の概要

本概要では、CIDP を適応とするエフガルチギモド PH20 SC の生物学的分析法に関する情報を記載する。健康被験者を対象とした臨床試験から得たエフガルチギモド PH20 SC の情報についても、提出及び承認済みの gMG IV CTD 又は gMG SC CTD から適宜引用する。

CIDP 被験者を対象とした下記の臨床試験で、エフガルチギモド PH20 SC の薬物動態、薬力学的作用及び免疫原性を評価するために用いた生物学的分析法は、2.7.1.1.3 に記載する。

- ARGX-113-1802 試験（以降本文中、試験名の「ARGX-113-」は省略）：ピボタル試験
- 1902 試験：1802 試験の非盲検継続投与試験
  - 生物学的分析のデータカットオフ日（2023 年 4 月 14 日）までに得られた中間データを含む。本試験は実施中のため、試験に特異的な生物学的分析法のパフォーマンスデータは得られていない。中間分析報告書は作成されていない。

健康被験者を対象とした下記の第 1 相試験で、エフガルチギモド PH20 SC の薬物動態、薬力学的作用及び免疫原性を評価するために用いた生物学的分析法は、提出及び承認済みの申請資料を引用する。

- 1901 試験（gMG SC CTD 2.7.1）
- 1907 試験（gMG SC CTD 2.7.1）
- 1702 試験（gMG IV CTD 2.7.1）

2.7.1.1.2　物理化学的性質と製剤開発の概要

エフガルチギモドは静脈内投与用注射製剤及び皮下投与用注射製剤として開発されており、本申請の対象は皮下投与用注射製剤であるエフガルチギモド PH20 SC である。物理化学的性質及び製剤開発については、gMG IV CTD（2.7.1.1.1）及び gMG SC CTD（2.7.1.1.1）に記載されている。製剤開発の詳細については、gMG SC CTD 3.2.P.2.2 に記載されている。

2.7.1.1.3　生物学的分析法の概要とバリデーション

2.7.1.1.3.1　薬物動態

血清中エフガルチギモド濃度は、エフガルチギモド PH20 SC の薬物動態を評価するために測定した。臨床で使用される用量では、rHuPH20 は循環血中で定量不能である（gMG SC CTD 5.3.5.3, HALO 10059）ため、エフガルチギモド PH20 SC の開発では、rHuPH20 の薬物動態は評価しなかった。

1802 試験及び 1902 試験の血清中エフガルチギモド濃度は、[redacted] システムを用いたサンドイッチ免疫学的測定法により測定した。中国で 1802 試験及び 1902 試験に参加した被験者の血清中エフガルチギモド濃度の測定には、電気化学発光免疫測定法（ECLIA）を用いた。

[redacted] 法は、ビオチン標識[redacted]でエフガルチギモドを捕捉し、蛍光標識[redacted]により検出する方法である。本分析法は、CIDP 患者の血清中での選択性を含めて、[redacted]社（旧[redacted]社又は旧[redacted]社）でバリデーションした（gMG SC CTD 5.3.1.4,

[redacted]327456QB10）。また、日本人被験者の血清中での選択性（gMG SC CTD 5.3.1.4, [redacted]349622QB10）及びヒト血清中のエフガルチギモドの長期安定性（5.3.1.4, [redacted]327456QB40）を評価するための補足バリデーションを行った。

ECLIA は、ビオチン標識[redacted]でエフガルチギモドを捕捉し、[redacted]標識[redacted]により検出する方法である。本分析法は[redacted]でバリデーションして（5.3.1.4, 8471-441）、中国人健康被験者から採取した血清（5.3.1.4, 8471-441-addendum01）及び中国人 CIDP 患者から採取した血清（5.3.1.4, 8478-707）中での選択性を評価するための補足バリデーションを行った。また、ヒト血清中のエフガルチギモドの 18 ヵ月までの長期安定性も評価した（5.3.1.4, 8471-441-addendum02）。

また、[redacted]法と ECLIA による薬物動態の結果がどのように関連しているかを示すため、分析法間のクロスバリデーションを申請者が評価した（5.3.1.4, ARGX-PTBA-0674）。

2 つの分析法について、分析法ライフサイクルの概要を表 2.7.1-1 に示す。血清中エフガルチギモド濃度の分析法バリデーション及び臨床試験での分析法パフォーマンスパラメータの詳細情報は表 2.7.1-2 及び表 2.7.1-3 に示す。また、クロスバリデーションに関する情報は表 2.7.1-4 に示す。

2.7.1.1.3.2　薬力学的作用

血清中総 IgG 濃度は、エフガルチギモド PH20 SC の薬力学的作用を評価するために測定した。

[redacted]プラットフォームを用いた血清中総 IgG 濃度測定のための免疫比濁アッセイキット（[redacted]社）は、[redacted]（5.3.1.4, VR-[redacted]-20[redacted]-05-383）、[redacted]（5.3.1.4, VR-[redacted]20[redacted]-12-452）及び[redacted]（5.3.1.4, VR-[redacted]-20[redacted]-11-705）で、臨床検査室改善法（Clinical Laboratory Improvement Amendments）及び米国病理医協会（College of American Pathologists）の要件に従ってバリデーションした。この体外診断用医薬品（IVD）アッセイについて、さらに CIDP 患者の血清での薬物干渉及び平行性のバリデーションを行った（5.3.1.4, VR-[redacted]-20[redacted]-9-687）。[redacted]でのアッセイは、1802 試験及び 1902 試験の血清サンプル分析に用いた。中国で 1802 試験及び 1902 試験に参加した被験者の総 IgG 測定には、[redacted]及び[redacted]でバリデーションした方法を用いた。

分析施設間の比較をする試験を[redacted]と[redacted]間（5.3.1.4, VR-[redacted]-20[redacted]-12-452）、及び[redacted]と[redacted]間（5.3.1.4, VR-[redacted]-20[redacted]-11-705）で実施し、薬力学的作用の測定方法が 3 つの分析施設間で同様であることを示した。

総 IgG の分析法について、分析法ライフサイクルの概要を表 2.7.1-5 に示す。血清中総 IgG 濃度の分析法バリデーション及び臨床試験での分析法パフォーマンスパラメータに関する詳細情報は表 2.7.1-6 に示す。

2.7.1.1.3.3　免疫原性

エフガルチギモド及び rHuPH20 の免疫原性は、1802 試験及び 1902 試験のベースライン及び治験薬投与後の血清及び血漿サンプルをそれぞれ用いて評価した。

エフガルチギモドに対する ADA 及び rHuPH20 に対する抗体は、3 段階の段階的方法を用いてスクリーニング、確認及び特徴付けを行った。

1. 全てのサンプルを ADA スクリーニングアッセイで分析した。
2. スクリーニングで陽性となったサンプルは、ADA 反応の特異性を評価するために ADA 確認アッセイで分析した。
3. 陽性が確認されたサンプルは、半定量的タイトレーションアッセイで分析した。

陽性が確認されたサンプルは、さらに中和抗体アッセイで中和活性を分析した。

rHuPH20 に対する中和抗体は、最初はスクリーニングアッセイと力価分析（すなわち 2 段階アプローチ）を用いて評価した。さらに、反応の特異性を決定するために、事後解析として中和抗体確認アッセイを実施した。

2.7.1.1.3.3.1　エフガルチギモドの免疫原性

1802 試験及び 1902 試験の血清サンプル中の ADA 測定には、Affinity Capture Elution（ACE）ブリッジング ELISA を使用した。

このアッセイは [redacted] 社（旧 [redacted] 社又は旧 [redacted] 社）でバリデーションして（5.3.1.4, [redacted]314331QB10）、新しい標識試薬を導入するため補足バリデーションを行った（5.3.1.4, [redacted]325305QB20）。日本人被験者の血清中での選択性（5.3.1.4, [redacted]349215QB10）及び CIDP 患者の血清中での選択性（5.3.1.4, [redacted]325305QB21）も評価した。

同じ分析法を [redacted] [redacted] でバリデーションして（5.3.1.4, 8471-440）、中国で 1802 試験及び 1902 試験に参加した被験者のエフガルチギモドに対する ADA 測定に使用した。

クロスバリデーションを [redacted] 社及び [redacted] [redacted] で行った（5.3.1.4, [redacted]353102QB10、5.3.1.4, 8471-440-addendum）。さらに、申請者は、2 つの分析施設での分析法パフォーマンスの相互比較を実施した（5.3.1.4, ARGX-PTBA-1001）。これらの評価により、ADA 分析法は 2 つの分析施設間で同様であることが確認された。

2 つの分析法について、分析法ライフサイクルの概要を表 2.7.1-7 に示す。分析法バリデーション及び臨床試験での分析法パフォーマンスパラメータに関する詳細情報は、表 2.7.1-8、表 2.7.1-9 及び表 2.7.1-10 に示す。また、クロスバリデーションに関する情報は表 2.7.1-11 に示す。

エフガルチギモドに対する ADA のスクリーニングアッセイ

ADA スクリーニングアッセイについては、Integrated Summary of Immunogenicity（ISI）（5.3.5.3, ARGX-113-9303-ISI, Section 3.2.1.1）に記載した。

エフガルチギモドに対する ADA の確認アッセイ

ADA 確認アッセイについては、ISI（5.3.5.3, ARGX-113-9303-ISI, Section 3.2.1.2）に記載した。

エフガルチギモドに対する ADA のタイトレーションアッセイ

ADA タイトレーションアッセイについては、ISI（5.3.5.3, ARGX-113-9303-ISI, Section 3.2.1.3）に記載した。

エフガルチギモドに対する中和抗体検出のためのアッセイ

エフガルチギモドに対する ADA 陽性が確認された全てのサンプルは、間接競合 ECLIA を用いて ADA の中和活性を分析した。本分析法には、アッセイの薬剤干渉耐性を改善するためのビオチン－薬剤抽出及び酸解離（BEAD）前処理のステップが含まれる。エフガルチギモドは、FcRn と野生型ヒト[redacted]標識 IgG3 との結合を阻害するが、エフガルチギモドに対する中和抗体はアッセイシグナルを回復する。中和抗体アッセイで観察されたアッセイシグナルの増加は、抗体の中和活性に比例する。このアッセイは、IgG のヒト FcRn への結合を拮抗阻害することにより、内因性 IgG の減少を促進するエフガルチギモドの主な作用機序をモニターするのに適した in vitro 分析法である（中和抗体アッセイの詳細については、5.3.5.3, ARGX-113-9303-ISI, Section 3.2.2 参照）。

1802 試験及び 1902 試験の血清サンプル中の中和抗体を測定するために使用したアッセイは、[redacted]社でバリデーションして CIDP 患者の血清中での選択性を評価した（5.3.1.4, [redacted]329686QB10）。日本人被験者の血清中での選択性を測定するために、補足バリデーションを行った（5.3.1.4, [redacted]349215QB10）。

同じ分析法を[redacted]でバリデーションして（5.3.1.4, 8471-439）、中国で 1802 試験及び 1902 試験に参加した被験者のエフガルチギモドに対する中和抗体の測定に使用した。

分析法ライフサイクルの概要を表 2.7.1-12 に示す。また、分析法バリデーション及び臨床試験での分析法パフォーマンスパラメータに関する詳細情報は表 2.7.1-13 及び表 2.7.1-14 に示す。

2.7.1.1.3.3.2　rHuPH20 の免疫原性

rHuPH20 に対する抗体を検出するために、電気化学発光ベースのブリッジング免疫アッセイが Halozyme 社により開発された。

このアッセイは[redacted]（旧[redacted]社）でバリデーションした（gMG SC CTD 5.3.1.4, HALO 20079 rev2）。CIDP 患者の血漿中での薬物干渉と選択性を評価するため、補足バリデーションを行った（gMG SC CTD 5.3.1.4, HALO 21111 rev1）。システム適合性限界値（5.3.1.4, HALO 22076 rev0）及び長期安定性仕様（5.3.1.4, HALO 23045 rev1）を更新するために追加のバリデーションを行った。本分析法は、1802 試験及び 1902 試験のサンプル分析に使用した。

同じ分析法を[redacted]でバリデーションして（5.3.1.4, HALO 21040 rev0）、中国で 1802 試験及び 1902 試験に参加した被験者の rHuPH20 に対する抗体の測定に使用した。

[redacted]及び[redacted]でクロスバリデーションを行い（5.3.1.4, HALO 22029 rev0）、2 つの分析施設間で免疫原性の分析法が同様であることを確認した。

2 つの分析法について、分析法ライフサイクルの概要を表 2.7.1-15 に示す。分析法バリデーション及び臨床試験での分析法パフォーマンスパラメータに関する詳細情報は、表 2.7.1-16～表 2.7.1-19 に示す。また、クロスバリデーションに関する情報は表 2.7.1-20 に示す。

rHuPH20 に対する抗体のスクリーニングアッセイ

スクリーニングアッセイについては、gMG SC CTD 5.3.5.3, ARGX-113-9023-ISI, Section 3.3.1.1 に記載されている。アッセイパラメータの詳細は ISI（5.3.5.3, ARGX-113-9303-ISI, Section 3.3.1.1）に記載した。

rHuPH20 に対する抗体の確認アッセイ

確認アッセイについては、ISI（5.3.5.3, ARGX-113-9303-ISI, Section 3.3.1.2）に記載した。

rHuPH20 に対する抗体のタイトレーションアッセイ

タイトレーションアッセイについては、ISI（5.3.5.3, ARGX-113-9303-ISI, Section 3.3.1.3）に記載した。

rHuPH20 に対する中和抗体検出のためのアッセイ

rHuPH20 に対する抗体の陽性が確認された全てのサンプルは、Halozyme 社独自のアッセイを用いて中和活性を分析した。本アッセイは、ヒアルロン酸を分解する rHuPH20 活性を阻害するヒト血漿中の中和抗体を検出するようにデザインされた。最初は、アッセイカットポイントを超える中和活性を示すサンプルを陽性とし、エンドポイント中和抗体力価を決定するために追加の分析を行った［2 段階アプローチ、スクリーニング及び力価分析（タイトレーションアッセイ）］。

1802 試験及び 1902 試験で使用した中和抗体分析法の説明は、gMG SC CTD 5.3.5.3, ARGX-113-9023-ISI, Section 3.3.2 に記載されている。

1802 試験及び 1902 試験のサンプルの分析には、Halozyme 社独自のアッセイ法を使用した。このアッセイは[redacted]でバリデーションした（gMG SC CTD 5.3.1.4, HALO 20078 rev3）。CIDP 患者の血漿中での薬物干渉及び選択性を評価するため、補足バリデーションを行った（gMG SC CTD 5.3.1.4, HALO 22047 rev0）。

同じ分析法を[redacted]でバリデーションして（5.3.1.4, HALO 21075 rev0）、中国で 1802 試験及び 1902 試験に参加した被験者の中和活性測定に使用した。

2 つの分析施設での結果を比較可能とするため、[redacted]と[redacted]でクロスバリデーションを行った（5.3.1.4, HALO 23115 rev0）。

1802 試験のステージ B の期間中、rHuPH20 に対する中和抗体の発現割合はエフガルチギモド PH20 SC 群で 4.5%であった。これは rHuPH20（Printz, 2022）及びエフガルチギモド PH20 SC（gMG SC CTD 5.3.5.1, ARGX-113-9023-ISI, Section 5.1.3.2）の臨床経験に基づいて予想された発現割合よりも高かったため、この結果を検討するために中和抗体を 3 段階アプローチ（スクリーニング、確認、力価分析）で評価する方法の一環として、追加の確認アッセイを事後的に実施した。スクリーニングアッセイで rHuPH20 に対する中和抗体が陽性であった検体を、さらに rHuPH20 に対する中和抗体確認アッセイで評価し、反応の特異性を判定した。確認アッセイは、rHuPH20 に対する中和抗体を除去するために、磁気ビーズに結合させた免疫グロブリン特異的遺伝子組換えタンパク質を用いる免疫除去工程である。

rHuPH20 に対する中和抗体の確認アッセイは、[redacted] 及び [redacted] でバリデーションした（それぞれ 5.3.1.4, HALO 24008 rev0、5.3.1.4, HALO 24028 rev0）。選択性は CIDP 患者の血漿中で評価した（5.3.1.4, HALO 24009 rev0）。

rHuPH20 に対する中和抗体のスクリーニング及びタイトレーションアッセイ、並びに確認アッセイについて、分析法ライフサイクルの概要を表 2.7.1-21 に示す。rHuPH20 に対する中和抗体スクリーニング及びタイトレーションアッセイの分析法バリデーション及び臨床試験での分析法パフォーマンスパラメータの詳細情報は表 2.7.1-22 及び表 2.7.1-23 に示す。また、クロスバリデーションに関する情報は表 2.7.1-24 に示す。rHuPH20 に対する中和抗体確認アッセイの分析法バリデーション及び臨床試験での分析法パフォーマンスパラメータの詳細情報は表 2.7.1-25 及び表 2.7.1-26 に示す。

2.7.1.2　個々の試験結果の要約

該当せず

2.7.1.3　全試験を通しての結果の比較と解析

該当せず

2.7.1.4　付録

以下の表には、エフガルチギモド PH20 SC の薬物動態、薬力学的作用及び免疫原性の評価のために 1802 試験及び 1902 試験で用いた生物学的分析法を記載する。gMG IV CTD 2.7.1 及び gMG SC CTD 2.7.1 に含めた分析報告書については、文章及び表中で参照する。

表 2.7.1-1　血清中エフガルチギモド定量の分析法ライフサイクル

	Method validation #3	Clinical study ARGX-113-1802	Method validation #5	Clinical study ARGX-113-1802[a]	Cross-validation methods #3 and #5
Analyte	Efgartigimod				
Validation type	Validation; supplemental validation and LTS	In-study	Validation; supplemental validation (LTS)	In-study	Cross-validation
eCTD reference number	[redacted]327456QB10, [redacted]349622QB10, [redacted]327456QB40	[redacted]325304QB20	8471-441 8471-441 addendum 01 8471-441 addendum 02 (LTS)	8478-707	[redacted]327456QB10, 8471-441 addendum 01, ARGX-PTBA-0674
Method ID	[redacted] version [redacted] to [redacted]	[redacted] version [redacted]	[redacted]	[redacted]	[redacted][b]: [redacted] version [redacted]: [redacted]
Duration of time method is in use	[redacted] 20[redacted] to present ([redacted]327456QB10) [redacted] 20[redacted] to present ([redacted]349622QB10) [redacted] 20[redacted] to present ([redacted]327456QB40)	[redacted] 20[redacted] to 20[redacted]	[redacted] 20[redacted] to present	[redacted] 20[redacted] to 20[redacted]	[redacted] 20[redacted] to present ([redacted]327456QB10) [redacted] 20[redacted] to present (8471-441 addendum 01)
Bioanalytical site	[redacted][b], [redacted], [redacted], UK		[redacted]		[redacted][b], [redacted], UK ([redacted]327456QB10) [redacted] (8471-441 addendum 01)

	Method validation #3	Clinical study ARGX-113-1802	Method validation #5	Clinical study ARGX-113-1802[a]	Cross-validation methods #3 and #5
Matrix	Human serum				
Platform	Sandwich immunoassay format using a [redacted] system		ECLIA		[redacted] vs ECLIA
Format	Sandwich immunoassay using [redacted] as capture and [redacted] coupled with [redacted] as detection reagent		Sandwich ECLIA using [redacted] as capture and [redacted] as detection reagent		Refer to validation reports [redacted]327456QB10 and 8471-441 addendum 01
Stock reference Batch/lot number Expiration date	Efgartigimod Batch/lot no.: [redacted] Exp. date: [redacted] 20 Batch/lot no: [redacted] Exp. date: [redacted] 20 Batch/lot no.: [redacted] Exp. date: [redacted] 20	Efgartigimod Batch/lot no.: [redacted] Exp. date: [redacted] 20	Efgartigimod Batch/lot no.: [redacted] Exp. date: [redacted] 20	Efgartigimod Batch/lot no.: [redacted] Exp. date: [redacted] 20	Refer to validation reports [redacted]327456QB10 and 8471-441 addendum 01
Calibration range from the LLOQ to the ULOQ	200-5000 ng/mL in 100% matrix		200-6000 ng/mL in 100% matrix		[redacted][b]: 200-10 000 ng/mL [redacted]: 200-6000 ng/mL
Matrix study population	Healthy, CIDP, ITP, MG, PV, healthy Japanese	CIDP	Healthy Chinese	CIDP	Healthy

	Method validation #3	Clinical study ARGX-113-1802	Method validation #5	Clinical study ARGX-113-1802[a]	Cross-validation methods #3 and #5
Link to reports and applicable amendments	gMG SC CTD, Module 5.3.1.4 [redacted]327456QB10 [redacted]349622QB10 Module 5.3.1.4 [redacted]327456QB40	Module 5.3.1.4 [redacted]325304QB20	Module 5.3.1.4 8471-441 8471-441 addendum 01 8471-441 addendum 02	Module 5.3.1.4 8478-707	gMG SC CTD, Module 5.3.1.4 [redacted]327456QB10 Module 5.3.1.4 8471-441 addendum 01 ARGX-PTBA-0674
Synopsis of amendment history	• Additional partial validation of truncated curve range due to unsuccessful bridging of high concentration batches (for drug batch [redacted]) • The ELISA and [redacted] assay to measure efgartigimod in serum were cross-validated	• Incurred sample reanalysis • Parallelism • Qualification of new QC and working stock	• The ECLIA and ELISA assay to measure efgartigimod in serum were cross-validated • Cross-validation with [redacted] QC and study samples from ARGX-113-1901 • LTS, selectivity in healthy individuals, working stock stability, new analyst(s), pooled serum, and new QC and calibrators	• Selectivity in CIDP study samples • Incurred sample reanalysis	NA

[redacted]=chimeric molecule consisting of an efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone [redacted] and a [redacted] tag; CIDP=chronic inflammatory demyelinating polyneuropathy; eCTD=electronic common technical document; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; ITP=immune thrombocytopenia; gMG=generalized myasthenia gravis; LLOQ=lower limit of quantification; LTS=long-term stability; NA=not applicable; no.=number; PV=pemphigus vulgaris; QC=quality control; SH=[redacted]; UK=United Kingdom; ULOQ=upper limit of quantification

a) This method was used to analyze samples from participants in mainland China.

b) [redacted] was formerly known as [redacted] or [redacted].

表 2.7.1-2　血清中エフガルチギモド定量の分析法パフォーマンスの要約－バリデーション
Method #3

Bioanalytical method validation report name	**Validation of a PK Assay for the Determination of ARGX-113 in Human Serum Using the [redacted] Platform (gMG SC CTD, Module 5.3.1.4, [redacted]327456QB10)** **Selectivity Testing of Japanese Sera Within the ARGX-113 PK Method (gMG SC CTD, Module 5.3.1.4, [redacted]349622QB10)** **Long-Term Stability of a PK Method for the Determination of ARGX-113 in Human Serum (Module** 5.3.1.4, [redacted]327456QB40)	
Method description	The method is a sandwich immunoassay format using a [redacted] Bioaffy system, with [redacted] as capture and [redacted] coupled with [redacted] to detect efgartigimod. Method validation details are included in the gMG SC CTD, Module 2.7.1, Table 6.	
Long-term storage	Stability of efgartigimod in human serum at [redacted] °C ± [redacted] °C and [redacted] °C ± [redacted] °C was demonstrated for up to [redacted] days.	[redacted]327456QB40, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12, Table 13, and Table 14
Method performance in study ARGX-113-1802 (Module 5.3.1.4, [redacted]325304QB20)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate)	[redacted]325304QB20, Section 7.4
Standard curve performance	Interassay bias range: [redacted]% to [redacted]% Interassay precision: [redacted]% CV	[redacted]325304QB20, Table 2
QC performance	Interassay bias range: [redacted]% to [redacted]% Interassay precision: [redacted]% CV	[redacted]325304QB20, Table 4
Method reproducibility	Incurred sample reanalysis was performed on [redacted] study samples, and [redacted]% met the prespecified criteria.	[redacted]325304QB20, Section 12.6
Parallelism	Parallelism is considered acceptable up to [redacted]-fold.	[redacted]325304QB20, Section 12.2.1
Prozone (hook effect)	The method is not susceptible to prozone in study sample dosing with ARGX-113.	[redacted]325304QB20, Section 12.2.1
Qualification runs	New quality control and working stock bridged.	[redacted]325304QB20, Section 12.2.2
Study sample analysis/stability	Stability of efgartigimod in human serum at [redacted] °C ± [redacted] °C was demonstrated for up to [redacted] days ([redacted]327456QB40). All analytical project samples were analyzed within the established stability period ([redacted] days) for ARGX-113 in human serum, except for [redacted] samples reported as NR.	[redacted]325304QB20, Section 7.4
Standard calibration curve performance during accuracy and precision runs	Calibration curve contains [redacted] standards from LLOQ to ULOQ	NA

[redacted]=chimeric molecule consisting of an efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone [redacted] and a [redacted] tag; ARGX-113=efgartigimod; CV=coefficient of variation; gMG=generalized myasthenia gravis; LLOQ=lower limit of quantification; NA=not applicable; NR=not reported; PK=pharmacokinetics; QC=quality control; SC=subcutaneous; ULOQ=upper limit of quantification

表 2.7.1-3　血清中エフガルチギモド定量の分析法パフォーマンスの要約－バリデーション
Method #5

Bioanalytical method validation report name	**Validation of a Method for the Determination of ARGX-113 in Human Serum Using Electrochemiluminescence (ECL) (Module** 5.3.1.4, 8471-441, 8471-441 addendum 01, and 8471-441 addendum 02)		
Method description	The method is an ECLIA with [redacted] as the capture antibody and [redacted] as the detection antibody.		
Materials used for standard calibration curve and concentration	Efgartigimod Batch/lot no.: [redacted] [redacted] mg/mL Exp. date: [redacted] 20[redacted]		
Validated assay range	200-6000 ng/mL in 100% matrix		
Material used for QCs and concentration	Efgartigimod Batch/lot no.: [redacted] [redacted] mg/mL Exp. date: [redacted] 20[redacted]		
MRD	1/[redacted]		
Source and lot of reagents	[redacted]: batch/lot [redacted], argenx [redacted]: batch/lot [redacted], argenx		
Regression model and weighting	4-parameter logistical model with weighting factor of 1/Y		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	[redacted] calibrators	8471-441, Table 1
	Interassay accuracy (%bias) from LLOQ to ULOQ[a]	[redacted]% to [redacted]%	8471-441, Table 1
	Cumulative precision (% CV) from LLOQ to ULOQ[a]	[redacted]%	8471-441, Table 1
Performance of QCs during accuracy and precision runs	**Intrarun accuracy (%bias) in [redacted] QCs**	LLOQ ([redacted] ng/mL) LQC ([redacted] ng/mL) MQC ([redacted] ng/mL) HQC ([redacted] ng/mL)	[redacted]% to [redacted]% [redacted]% to [redacted]% [redacted]% to [redacted]% [redacted]% to [redacted]%	8471-441, Table 3

Bioanalytical method validation report name	Validation of a Method for the Determination of ARGX-113 in Human Serum Using Electrochemiluminescence (ECL) (Module 5.3.1.4, 8471-441, 8471-441 addendum 01, and 8471-441 addendum 02)		
		ULOQ ([redacted] ng/mL)	[redacted]% to [redacted]%	
	Interbatch precision (%CV)	LLOQ ([redacted] ng/mL) LQC ([redacted] ng/mL) MQC ([redacted] ng/mL) HQC ([redacted] ng/mL) ULOQ ([redacted] ng/mL)	[redacted]% [redacted]% [redacted]% [redacted]% [redacted]%	8471-441, Table 3
	Total error	LLOQ ([redacted] ng/mL) LQC ([redacted] ng/mL) MQC ([redacted] ng/mL) HQC ([redacted] ng/mL) ULOQ ([redacted] ng/mL)	[redacted]% [redacted]% [redacted]% [redacted]% [redacted]%	8471-441, Table 3
Selectivity and matrix effect	[redacted] lots of individual healthy non-Chinese sera Observed bias at LLOQ concentration: [redacted]% to [redacted]% Observed bias at ULOQ concentration: [redacted]% to [redacted]% ([redacted] lot >ULOQ) All unspiked sera were determined below the LLOQ of the assay, [redacted]% of the spiked matrixes were determined within [redacted]% of the LLOQ concentration, and [redacted]% of the spiked matrixes were determined within [redacted]% of the ULOQ concentration.			8471-441, Table 9
	[redacted] lots of individual healthy Chinese sera Observed bias at LLOQ concentration: [redacted]% to [redacted]% Observed bias at ULOQ concentration: [redacted]% to [redacted]% All unspiked sera were determined below the LLOQ of the assay, [redacted]% of the spiked matrixes were determined within [redacted]% of the LLOQ concentration, and [redacted]% of the spiked matrixes were determined within [redacted]% of the ULOQ concentration.			8471-441 addendum01, Table 6
Interference and specificity	Interference and specificity were not tested during this validation.			NA
Hemolysis effect	[redacted] hemolytic lots prepared with individual healthy sera Observed bias at LLOQ concentration: [redacted]% to [redacted]% Observed bias at ULOQ concentration: [redacted]% to [redacted]% All unspiked sera were determined below the LLOQ of the assay, [redacted]% of the spiked matrixes were determined within [redacted]% of the LLOQ concentration, and [redacted]% of the spiked matrixes were determined within [redacted]% of the ULOQ concentration.			8471-441, Table 10
Lipemic effect	[redacted] lipemic lots prepared with individual healthy sera Observed bias at LLOQ concentration: [redacted]% to [redacted]% Observed bias at ULOQ concentration: [redacted]% to [redacted]% All unspiked sera were determined below LLOQ of the assay, [redacted]% of the spiked matrixes were determined within [redacted]% of the LLOQ concentration, and [redacted]% of the spiked matrixes were determined within [redacted]% of the ULOQ concentration.			8471-441, Table 11

Bioanalytical method validation report name	**Validation of a Method for the Determination of ARGX-113 in Human Serum Using Electrochemiluminescence (ECL) (Module** 5.3.1.4, 8471-441, 8471-441 addendum 01, and 8471-441 addendum 02)	
Dilution linearity and hook effect	Dilution linearity was tested with [redacted] dilution factors up to 1/[redacted]. The bias observed at a dilution of 1/[redacted] was –[redacted]% to [redacted]. No hook effect was observed.	8471-441, Table 12
Bench-top/process stability	[redacted] hours at room temperature	8471-441, Table 15 and Table 16
Refrigerator Stability	[redacted] hours [redacted] minutes at [redacted] °C to [redacted] °C	8471-441, Table 17 and Table 18
Freeze-thaw stability	[redacted] cycles at [redacted] °C to [redacted] °C and [redacted] cycles at [redacted] °C to [redacted] °C	8471-441, Table 13 and Table 14
Long-term storage	[redacted] days at [redacted] °C to [redacted] °C and [redacted] days at [redacted] °C to −10 °C [redacted] days at [redacted] °C to [redacted] °C and [redacted] days at [redacted] °C to [redacted] °C	8471-441 addendum 01, Table 12 and Table 13 8471-441 addendum 02, Table 7 and Table 8
Working stock stability	[redacted] days at [redacted] °C to [redacted] °C All stability results were within the preset acceptance criteria.	8471-441 addendum 01, Table 14 and Table 15
Robustness	Coating incubation times were validated between [redacted] and [redacted] minutes. Sample incubation times were validated between [redacted] and [redacted] minutes. Detection antibody incubation times were validated between [redacted] and [redacted] minutes.	8471-441, Table 6
Parallelism	NA	NA
Carry over	NA	NA
Reference material bridging	NA	NA
Method performance in study ARGX-113-1802 (Module 5.3.1.4, 8478-707)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate)	8478-707, Section 14.1
Standard curve performance	Interassay bias range: [redacted]% to [redacted]% Interassay precision: [redacted]% CV	8478-707, Table 2
QC performance	Interassay bias range: [redacted]% to [redacted]% Interassay precision: [redacted]% CV	8478-707, Table 6

Bioanalytical method validation report name	**Validation of a Method for the Determination of ARGX-113 in Human Serum Using Electrochemiluminescence (ECL) (Module** 5.3.1.4, 8471-441, 8471-441 addendum 01, and 8471-441 addendum 02**)**	
Method reproducibility	Incurred sample reanalysis was performed on [redacted] study samples, and [redacted]% met the prespecified criteria.	8478-707, Section 14.4
Selectivity	[redacted] lots of sera from Chinese patients with CIDP Observed bias at LLOQ concentration: [redacted]% to [redacted]% Observed bias at HQC concentration: [redacted]% to [redacted]% All unspiked sera were determined below the LLOQ of the assay, [redacted]% of the spiked matrixes were determined within [redacted]% of the LLOQ concentration and [redacted]% of the spiked matrixes were determined within [redacted]% of the ULOQ concentration.	8478-707, Table 11
Study sample analysis/ stability	Stability of efgartigimod in human serum at [redacted]°C to [redacted] °C was demonstrated for up to [redacted] days. This stability period covers the maximum length of time from specimen collection to analysis (ie, [redacted] days).	8478-707, Section 13.3.1
Standard calibration curve performance during accuracy and precision runs	Calibration curve contains [redacted] standards from LLOQ to ULOQ	NA

ARGX-113=efgartigimod; CV=coefficient of variation; ECLIA=electrochemiluminescence immunoassay; HQC=high quality control; LLOQ=lower limit of quantification; LQC=low quality control; MQC=mid quality control; MRD=minimum required dilution; NA=not applicable; PK=pharmacokinetics; QC=quality control; ULOQ=upper limit of quantification

a) Data presented is from all accepted validation runs.

表 2.7.1-4　血清中エフガルチギモド定量の分析法パフォーマンスの要約－Method #3 と Method #5 のクロスバリデーション

Bioanalytical method validation report name and hyperlink	**Validation of a PK Assay for the Determination of ARGX-113 in Human Serum Using the [redacted] Platform (gMG SC CTD, Module 5.3.1.4, [redacted]327456QB10)** **Validation of a Method for the Determination of ARGX-113 in Human Serum Using Electrochemiluminescence (ECL) (Module** 5.3.1.4, 8471-441 addendum 01) **Statistical Analysis of PK Cross-Validation Data for Efgartigimod Obtained With the [redacted] and ECLIA Methods at [redacted] and [redacted], Respectively (Module** 5.3.1.4, ARGX-PTBA-0674)				
Changes in method	Comparison of [redacted] method ([redacted][a], validated in report [redacted]327456QB10) with ECLIA method ([redacted], validated in report 8471-441 addendum 01)				
New validated assay range, if any	Range [redacted][a]: 200-10 000 ng/mL ([redacted]327456QB10) Range [redacted]: 200-6000 ng/mL (8471-441)				
Validation parameters	**Cross-validation performance**				**Source location**
Standard calibration curve performance during accuracy and precision runs	**Cumulative accuracy (%bias) in standard calibrators from LLOQ to ULOQ**	[redacted][a] Interrun accuracy	[redacted]% to [redacted]%		[redacted]327456QB10, Table 10
		[redacted] Interrun accuracy	[redacted]% to [redacted]%		8471-441 addendum01, Table 1
	Cumulative precision (%CV) from LLOQ to ULOQ	[redacted][a] Interrun precision	[redacted]%		[redacted]327456QB10, Table 10
		[redacted] Interrun precision	[redacted]%		8471-441 addendum 01, Table 1
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in [redacted] QCs**	[redacted][a] Interrun accuracy	LLOQ ([redacted] ng/mL) LQC ([redacted] ng/mL) MQC ([redacted] ng/mL) HQC ([redacted] ng/mL) ULOQ ([redacted] ng/mL)	[redacted]% [redacted]% [redacted]% [redacted]% [redacted]%	[redacted]327456QB10, Table 55
		[redacted] Interrun accuracy	LLOQ LQC ([redacted] ng/mL) MQC ([redacted] ng/mL) HQC ([redacted] ng/mL) ULOQ	NA [redacted]% [redacted]% [redacted]% NA	8471-441 addendum 01, Table 4

Bioanalytical method validation report name and hyperlink	**Validation of a PK Assay for the Determination of ARGX-113 in Human Serum Using the [redacted] Platform (gMG SC CTD, Module 5.3.1.4, [redacted]327456QB10)** **Validation of a Method for the Determination of ARGX-113 in Human Serum Using Electrochemiluminescence (ECL) (Module** 5.3.1.4, 8471-441 addendum 01) **Statistical Analysis of PK Cross-Validation Data for Efgartigimod Obtained With the [redacted] and ECLIA Methods at [redacted] and [redacted], Respectively (Module** 5.3.1.4, ARGX-PTBA-0674)				
Performance of QCs during accuracy and precision runs (continued)	**Precision (%CV)**	[redacted][a] Interrun precision	LLOQ ([redacted] ng/mL) LQC ([redacted] ng/mL) MQC ([redacted] ng/mL) HQC ([redacted] ng/mL) ULOQ ([redacted] ng/mL)	[redacted]% [redacted]% [redacted]% [redacted]% [redacted]%	[redacted]327456QB10, Table 55
		[redacted] Interrun precision	LLOQ LQC ([redacted] ng/mL) MQC ([redacted] ng/mL) HQC ([redacted] ng/mL) ULOQ	NA [redacted]% [redacted]% [redacted]% NA	8471-441 addendum 01, Table 4
	TE	[redacted][a]	LLOQ ([redacted] ng/mL) LQC ([redacted] ng/mL) MQC ([redacted] ng/mL) HQC ([redacted] ng/mL) ULOQ ([redacted] ng/mL)	[redacted]% [redacted]% [redacted]% [redacted]% [redacted]%	[redacted]327456QB10, Table 55
		[redacted]	LLOQ LQC ([redacted] ng/mL) MQC ([redacted] ng/mL) HQC ([redacted] ng/mL) ULOQ	NA [redacted]% [redacted]% [redacted]% NA	8471-441 addendum 01, Table 4
Cross-validation	[redacted] incurred samples	All concentrations (n=[redacted], [redacted] sample in ECLIA was BLQ)	Average difference of [redacted]%; [redacted] ([redacted]%) incurred samples within ±[redacted]% difference		ARGX-PTBA-0674, Table 4, Table 5, Table 6, and Table 7
		Excluding [redacted] extreme observations (n=[redacted])	Average difference of [redacted]%; [redacted] ([redacted]%) incurred samples within ±[redacted]% difference		ARGX-PTBA-0674, Table 4, Table 5, Table 6, and Table 7

Bioanalytical method validation report name and hyperlink	**Validation of a PK Assay for the Determination of ARGX-113 in Human Serum Using the [redacted] Platform (gMG SC CTD, Module 5.3.1.4, [redacted]327456QB10)** **Validation of a Method for the Determination of ARGX-113 in Human Serum Using Electrochemiluminescence (ECL) (Module** 5.3.1.4, 8471-441 addendum 01) **Statistical Analysis of PK Cross-Validation Data for Efgartigimod Obtained With the [redacted] and ECLIA Methods at [redacted] and [redacted], Respectively (Module** 5.3.1.4, ARGX-PTBA-0674)		
	[redacted] cross-validation QC samples	[redacted] QCs spiked with range of [redacted] to [redacted] ng/mL [redacted][a] ([redacted]) accuracy: [redacted]% to [redacted]% [redacted] (ECLIA): accuracy [redacted]% to [redacted]% ([redacted] out of [redacted] did not meet criteria) % difference [redacted] vs ECLIA: [redacted]% to [redacted]%	ARGX-PTBA-0674, Table 3

BLQ=below limit of quantification; CV=coefficient of variation; ECLIA=electrochemiluminescence immunoassay; HQC=high quality control; LLOQ=lower limit of quantification; LQC=low quality control; MQC=mid quality control; NA=not applicable; PK=pharmacokinetics; QC=quality control; SH=[redacted]; TE=total error; ULOQ=upper limit of quantification
a) [redacted] was formerly known as [redacted] or [redacted].

表 2.7.1-5　血清中総 IgG 定量の分析法ライフサイクル

	Method validation #3	Clinical study ARGX-113-1802
Analyte	Total IgG	
Validation type	Primary validation, secondary validation, LTS, and supplemental validation	In-study
eCTD reference number	Primary validation[a]: VR-[redacted]-20[redacted]-05-383 Secondary validation[a]: VR-[redacted]-20[redacted]-12-452 Secondary validation[a]: VR-[redacted]-20[redacted]-11-705 Supplemental validation: VR-[redacted]-20[redacted]-9-687	AR-CL-SH-SZ-20[redacted]-08-151 (ARGX0013) AR-CL-US-20[redacted]-08-137 (ARGX0001)
Method ID	[redacted] Package Insert	[redacted] Package Insert
Duration of time method is in use	[redacted] 20[redacted] to present	[redacted] 20[redacted] to [redacted] 20[redacted]
Bioanalytical sites	• [redacted] US • [redacted] People's Republic of China • [redacted] People's Republic of China	
Matrix	Human serum	
Platform	Immunoturbidimetric assay (IVD test)	
Format	Antibodies react with antigens in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically.	
Stock reference **Lot number** **Expiration date**	Refer to validation reports.	[redacted] Batch/lot no: refer to analysis reports
Calibration range from the LCS to the HCS	30-3900 mg/dL	
Matrix study population	Healthy, ITP, PV, MG, CIDP	CIDP

	Method validation #3	Clinical study ARGX-113-1802
Link to reports and applicable amendments	Module 5.3.1.4, VR-[redacted]-20[redacted]-05-383, VR-[redacted]-20[redacted]-12-452, VR-[redacted]-20[redacted]-11-705, VR-[redacted]-20[redacted]-9-687	Module 5.3.1.4, AR-CL-SH-SZ-20[redacted]-08-151 (ARGX0013), AR-CL-US-20[redacted]-08-137 (ARGX0001)
Synopsis of amendment history	NA	NA

CIDP=chronic inflammatory demyelinating polyneuropathy; eCTD=electronic common technical document; ELISA=enzyme-linked immunosorbent assay; Exp=expiration; gMG=generalized myasthenia gravis; IgG=immunoglobulin γ; ITP=immune thrombocytopenia; IVD=in vitro diagnostic; [redacted]=[redacted]; LLOQ=lower limit of quantification; LTS=long-term stability; NA=not applicable; PV=pemphigus vulgaris; QC=quality control; SH=[redacted]; SZ=[redacted]; ULOQ=upper limit of quantification; US=United States

a) Primary and secondary validations are the property of [redacted].

表 2.7.1-6　血清中総 IgG 定量の分析法パフォーマンスの要約－バリデーション Method #3

Bioanalytical method validation report name	**Primary validation:** **Immunoglobulins G, A, M (IgG, IgA, IgM), Transferrin, Complement C3, Complement C4, and Haptoglobin in Serum by Immunoturbidimetric Assay ([redacted]) on [redacted]** **(Module** 5.3.1.4, VR-[redacted]-20[redacted]-05-383) **Client validation addendum summary:** **Immunoglobulins G (IgG) in Serum by Immunoturbidimetric Assay ([redacted]) on [redacted]** **(Module** 5.3.1.4, VR-[redacted]-20[redacted]-9-687) **Secondary validation:** **Immunoglobulins G, A, M (IgG, IgA, IgM) in Serum by Immunoturbidimetric Assay ([redacted]) on [redacted]** **(Module** 5.3.1.4, VR-[redacted]-20[redacted]-12-452) **Secondary validation:** **Immunoglobulins G, A, M (IgG, IgA, IgM), Transferrin, Haptoglobin, Complement C3, Complement C4, Apolipoprotein A1 (Apo A1), Apolipoprotein B (Apo B), And Rheumatoid Factor (RF II) by Immunoturbidimetric assay on [redacted] Module** **(Module** 5.3.1.4, VR-[redacted]-20[redacted]-11-705)	
Method description	The assay to determine the total IgG concentration is a quantitative IVD kit based on immunoturbidimetry performed at [redacted], [redacted], and [redacted]. Antibodies react with antigens in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically.	
Reportable range	30-3900 mg/dL	
Validation parameters	**Supplemental validation**	**Source location**
Interference and specificity	Samples from [redacted] healthy participants were spiked with efgartigimod [redacted] µg/mL (C_{max}) (run in [redacted] replicates). There was no interference of efgartigimod up to [redacted] µg/mL. The observed bias ranged from [redacted]% to [redacted]%; [redacted]% of samples passed with bias [redacted]%.	VR-[redacted]-20[redacted]-9-687, Table 4
Parallelism	Serial dilution of [redacted] healthy human sera was performed to validate manual dilution (neat, [redacted]×, [redacted]×, [redacted]×) on top of on-board dilution. Acceptable parallelism was seen up to [redacted]-fold manual dilution. Serial dilution of [redacted] sera from patients with MG was performed to validate manual dilution (neat, [redacted]×, [redacted]×, [redacted]×) on top of on-board dilution. Acceptable parallelism was seen up to [redacted]-fold manual dilution. Serial dilution of [redacted] sera from patients with PV was performed to validate manual dilution (neat, [redacted]×, [redacted]×, [redacted]×) on top of on-board dilution. Acceptable parallelism was seen up to [redacted]-fold manual dilution.	VR-[redacted]-20[redacted]-9-687, Table 6, Table 8, Table 9, Table 10, and Table 11
Parallelism (continued)	Serial dilution of [redacted] sera from patients with CIDP was performed to validate manual dilution (neat, [redacted]×, [redacted]×, [redacted]×) on top of on-board dilution. Acceptable parallelism was seen up to [redacted]-fold manual dilution. Serial dilution of [redacted] sera from patients with ITP was performed to validate manual dilution (neat, [redacted]×, [redacted]×, [redacted]×) on top of on-board dilution. Acceptable parallelism was seen up to [redacted]-fold manual dilution.	VR-[redacted]-20[redacted]-9-687, Table 6, Table 8, Table 9, Table 10, and Table 11

Bioanalytical method validation report name	**Primary validation:** **Immunoglobulins G, A, M (IgG, IgA, IgM), Transferrin, Complement C3, Complement C4, and Haptoglobin in Serum by Immunoturbidimetric Assay ([redacted]) on [redacted]** **(Module** 5.3.1.4, VR-[redacted]-20[redacted]-05-383) **Client validation addendum summary:** **Immunoglobulins G (IgG) in Serum by Immunoturbidimetric Assay ([redacted]) on [redacted]** **(Module** 5.3.1.4, VR-[redacted]-20[redacted]-9-687) **Secondary validation:** **Immunoglobulins G, A, M (IgG, IgA, IgM) in Serum by Immunoturbidimetric Assay ([redacted]) on [redacted]** **(Module** 5.3.1.4, VR-[redacted]-20[redacted]-12-452) **Secondary validation:** **Immunoglobulins G, A, M (IgG, IgA, IgM), Transferrin, Haptoglobin, Complement C3, Complement C4, Apolipoprotein A1 (Apo A1), Apolipoprotein B (Apo B), And Rheumatoid Factor (RF II) by Immunoturbidimetric assay on [redacted] Module** **(Module** 5.3.1.4, VR-[redacted]-20[redacted]-11-705)	
Method performance in study ARGX-113-1802 **(Module** 5.3.1.4, AR-CL-SH-SZ-20[redacted]-08-151 **[ARGX0013] and** AR-CL-US-20[redacted]-08-137 **[ARGX0001])**		
Assay passing rate	All quality controls for all sample runs were within acceptance limits. All results met internal and external acceptance criteria.	AR-CL-SH-SZ-20[redacted]-08-151, Section 10 AR-CL-US-20[redacted]-08-137, Section 10

CIDP=chronic inflammatory demyelinating polyneuropathy; Cmax=maximal concentration; IgG=immunoglobulin γ; ITP=immune thrombocytopenia; IVD=in vitro diagnostic; MG=myasthenia gravis; PV=pemphigus vulgaris; SH=[redacted]; SZ=[redacted]; US=United States

表 2.7.1-7　エフガルチギモドに対する ADA 検出のための分析法ライフサイクル

	Validation method #3	Supplemental validation method #3	Clinical study ARGX-113-1802	Validation method #5	Clinical study ARGX-113-1802[a]	Cross-validation methods #3 and #5
Analyte	ADA against efgartigimod					
Validation type	Validation	Supplemental validations	In-study	Validation	In-study	Cross-validation
eCTD reference number	[redacted]314331QB10	[redacted]325305QB20, 349215QB10	[redacted]325305QB21	8471-440, 8471-440 addendum	8478-707	[redacted]353102QB10, 8471-440 addendum, ARGX-PTBA-1001
Method ID	[redacted] version [redacted]	[redacted] version [redacted] ([redacted]325305QB20) [redacted] version [redacted] ([redacted]349215QB10)	[redacted] version [redacted]	[redacted]	[redacted]	[redacted][b]; [redacted] version [redacted]; [redacted]
Duration of time method is in use	[redacted] 20[redacted] to present	[redacted] 20[redacted] to present ([redacted]325305QB20) [redacted] 20[redacted] to present ([redacted]349215QB10)	[redacted] to 20[redacted]	[redacted] 20[redacted] to present	[redacted] 20[redacted] to 20[redacted]	[redacted][b]; [redacted] 20[redacted] to present; [redacted] 20[redacted] to present
Bioanalytical site	[redacted][b], [redacted], [redacted], [redacted], [redacted], UK			[redacted]		[redacted][b], [redacted], [redacted], UK ([redacted]353102QB10) [redacted] (8471-440)
Matrix	Human serum					

	Validation method #3	Supplemental validation method #3	Clinical study ARGX-113-1802	Validation method #5	Clinical study ARGX-113-1802[a]	Cross-validation methods #3 and #5
Platform	ELISA					
Format	ACE bridging format					
Stock reference Lot number Expiration date	**Efgartigimod:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** 2D11hFc Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]	**Efgartigimod:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** 2D11hFc Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]	**Efgartigimod:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** 2D11hFc Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]	**Efgartigimod:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** 2D11hFc Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] Polyclonal rabbit anti-ARGX-113 (PC2) Batch/lot no: [redacted] Exp. date: [redacted] 20[redacted]	**Efgartigimod:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** 2D11hFc Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]	**Efgartigimod:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** 2D11hFc Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]
Matrix study population	Healthy, ITP	Healthy, Japanese	CIDP	Healthy, Chinese	CIDP	Healthy
Link to reports and applicable amendments	Module 5.3.1.4, [redacted]314331QB10	Module 5.3.1.4, [redacted]325305QB20[redacted] [redacted]349215QB10	Module 5.3.1.4, [redacted]325305QB21	Module 5.3.4.1, 8471-440	Module 5.3.1.4, 8478-707	Module 5.3.4.1, [redacted]353102QB10, 8471-440 addendum, ARGX-PTBA-1001

	Validation method #3	Supplemental validation method #3	Clinical study ARGX-113-1802	Validation method #5	Clinical study ARGX-113-1802[a]	Cross-validation methods #3 and #5
Synopsis of amendment history	NA	Qualification of new batch of efgartigimod (batch [redacted])	NA	NA	NA	NA

2D11hFc=chimeric molecule consisting of efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone 2D11 and a human Fc; ACE=affinity capture elution; ADA=antidrug antibody(ies); CIDP=chronic inflammatory demyelinating polyneuropathy; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; ITP=immune thrombocytopenia; NA=not applicable; no.=number; PC=positive control; UK=United Kingdom

a) This method was used to analyze samples from participants in mainland China.

b) [redacted] was formerly known as [redacted] or [redacted].

表 2.7.1-8　エフガルチギモドに対する ADA 検出のための分析法パフォーマンスの要約－バリデーション Method #3

Bioanalytical method validation report name	**Validation of an Immunogenicity Assay for the Determination of Anti-ARGX-113 Antibodies in Human Serum (Module** 5.3.1.4, [redacted]314331QB10**)**			
Method description	ADA against efgartigimod were detected with an ACE bridging ELISA on the [redacted] platform.			
Material used for QCs	PC antibody: 2D11hFc Batch/lot no.: [redacted] Concentration: [redacted] mg/mL			
Source and lot of reagents	Efgartigimod, batch/lot no.: [redacted]			
MRD	Samples are tested undiluted (MRD=1)			
Validation parameters	**Method validation summary**			**Source location**
SCP factor determination	A multiplicative SCP factor of [redacted] was determined with sera from [redacted] healthy participants, ensuring a [redacted]% false positive error rate. The same CPF is used for the ADA titration assay.			[redacted]314331QB10, Sections 6.1 and 11.5
CCP determination	CCP was calculated as a floating confirmatory ratio of [redacted] with [redacted] sera from healthy participants, ensuring a [redacted]% false positive error rate. The drug concentration in the confirmatory tier is [redacted] µg/mL efgartigimod.			[redacted]314331QB10, Sections 6.1 and 11.6
Sensitivity	Estimated sensitivity for screening was calculated at [redacted] ng/mL at [redacted]% CI. Estimated sensitivity for confirmatory was interpolated at [redacted] ng/mL at [redacted]% CI.			[redacted]314331QB10, Table 2
Selectivity	In healthy individuals: [redacted] ([redacted]%) individuals scored positive at an LPC level of [redacted] ng/mL in screening and confirmatory assay. In patients with ITP: [redacted] ([redacted]%) individuals scored positive at an LPC level of [redacted] ng/mL in screening and confirmatory assay.			[redacted]314331QB10, Table 10 [redacted]314331QB10, Table 11
Titer precision	All endpoint titer values of individuals and HPC are within [redacted] dilution step from the median. An MSR of [redacted] was determined from the interpolated titers of individuals.			[redacted]314331QB10, Table 13, Sections 6.1 and 11.13
QC concentrations	HPC: [redacted] ng/mL MPC: [redacted] ng/mL LPC: [redacted] ng/mL			[redacted]314331QB10, Table 4, Table 5, Table 6, Table 7, and Table 8
Precision of QCs	**Screening assay**	**Intraassay %CV (response)**	[redacted]% for HPC, MPC, LPC, and NC	[redacted]314331QB10, Table 4, Table 5, Table 6, Table 7,

Bioanalytical method validation report name	**Validation of an Immunogenicity Assay for the Determination of Anti-ARGX-113 Antibodies in Human Serum (Module** 5.3.1.4, LGC314331QB10)			
		Interassay %CV (norm response)	[redacted]% for HPC, MPC, and LPC	and Table 8
		Interassay %CV (response)	[redacted]% for NC	
	Confirmatory assay	**Intraassay %CV (response)**	[redacted]% for HPC, MPC, LPC, and NC	
		Interassay %CV (response)	[redacted]% for HPC, MPC, LPC, and NC	
System suitability ranges	HPC ([redacted] ng/mL) MPC ([redacted] ng/mL) LPC ([redacted] ng/mL) NC (OD)	[redacted] ≤ HPC/NC ≤ [redacted] [redacted] ≤ MPC/NC ≤ [redacted] LPC/NC ≤ [redacted] OD NC ≤ [redacted]		[redacted]314331QB10, Table 5, Table 6, Table 7, and Table 8
Drug tolerance	Mean drug tolerance at [redacted] ng/mL of PC: [redacted] µg/mL No drug tolerance observed at [redacted] ng/mL of the PC			[redacted]314331QB10, Table 9
Hook effect	No hook effect was observed up to [redacted] ng/mL of the PC.			[redacted]314331QB10, Table 3
Hemolysis effect	No hemolytic effect. LPC ([redacted] ng/mL) scored positive in [redacted] ([redacted]%) hemolytic samples in the screening and confirmatory assay.			[redacted]314331QB10, Table 12
Lipemic effect	No lipemic effect. LPC ([redacted] ng/mL) scored positive in [redacted] ([redacted]%) lipemic samples in the screening and confirmatory assay.			[redacted]314331QB10, Table 12
Bench-top/process stability	Up to [redacted] hours.			[redacted]314331QB10, Table 14
Freeze-thaw stability	Up to [redacted] freeze-thaw cycles at [redacted] °C ± [redacted] °C.			[redacted]314331QB10, Table 14
Long-term storage	Up to [redacted] days at [redacted] °C and [redacted] °C.			[redacted]314331QB10, Table 15

2D11hFc=chimeric molecule consisting of efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone 2D11 and an Fc; ACE=affinity capture elution; ADA=antidrug antibody(ies); CCP=confirmatory cut point; CP=cut point; CPF=cut point factor; CV=coefficient of variation; ECL=electrochemiluminescence; ELISA=enzyme-linked immunosorbent assay; EPT=endpoint titer; Fc=crystallizable fragment; FPR=false positive rate; HPC=high positive control; ITP=immune thrombocytopenia; LPC=low positive control; MPC=medium positive control; MRD=minimum required dilution; [redacted]; MSR=minimum significant ratio; NC=negative control; no.=number; OD=optical density; PC=positive control; QC=quality control; SCP=screening cut point

表 2.7.1-9　エフガルチギモドに対する ADA 検出のための分析法パフォーマンスの要約－補足バリデーション Method #3

Bioanalytical method validation report name	**Supplemental Validation of a Method for the Determination of Anti-ARGX-113 Antibodies in Human Serum (Module** 5.3.1.4, [redacted]325305QB20) **Supplemental Method Validation for Selectivity Assessment in Japanese Sera Within the ARGX-113 (ELISA) and NAb ([redacted] ECL) Methods (Module** 5.3.1.4, [redacted]349215QB10)			
Method description	ADA against efgartigimod were detected with an ACE bridging ELISA on the [redacted] platform.			
Material used for QCs	2D11hFc Batch/lot no.: [redacted] Concentration: [redacted] mg/mL			
Source and lot of reagents	Efgartigimod, batch/lot no.: [redacted] Efgartigimod, batch/lot no.: [redacted]			
MRD	Samples are tested undiluted (MRD=1)			
Validation parameters	**Method validation summary**[a]			**Source location**
SCP factor determination	A multiplicative screening CPF of [redacted] was determined with sera from [redacted] healthy participants to target a [redacted]% false positive error rate. The same CPF is used for the ADA titration assay.			[redacted]325305QB20, Section 10.3
CCP determination	The CCP was calculated as a fixed inhibition percentage of [redacted]%, with [redacted] sera from healthy participants, to ensure a [redacted]% false positive error rate. The drug concentration in the confirmatory tier is [redacted] µg/mL efgartigimod.			[redacted]325305QB20, Section 10.4
Sensitivity	Estimated sensitivity for screening was calculated at [redacted] ng/mL at [redacted]% CI. Estimated sensitivity for confirmatory was interpolated at [redacted] ng/mL at [redacted]% CI.			[redacted]325305QB20, Table 2
Selectivity	In healthy Japanese individuals, [redacted] ([redacted]%) individuals were positive at the LPC level of [redacted] ng/mL in screening and confirmatory assays.			[redacted]349215QB10, Section 9.1
Titer precision	All titer samples had the same EPT result.			[redacted]325305QB20, Section 10.10
QC concentrations	HPC: [redacted] ng/mL MPC: [redacted] ng/mL LPC: [redacted] ng/mL NC: [redacted] ng/mL			[redacted]325305QB20, Table 3, Table 4, Table 5, and Table 6
Precision of QCs	**Screening assay**	**Intraassay %CV (response)**	[redacted]% for HPC, MPC, LPC, and NC	
		Interassay %CV (norm response)	[redacted]% for HPC and LPC, [redacted]% for MPC	

Bioanalytical method validation report name	**Supplemental Validation of a Method for the Determination of Anti-ARGX-113 Antibodies in Human Serum (Module** 5.3.1.4, [redacted]325305QB20**)** **Supplemental Method Validation for Selectivity Assessment in Japanese Sera Within the ARGX-113 (ELISA) and NAb ([redacted] ECL) Methods (Module** 5.3.1.4, [redacted]349215QB10**)**			
		Interassay %CV (response)	[redacted]% for NC	
	Confirmatory assay	**Intraassay %CV (response)**	[redacted]% for HPC, MPC, LPC, and NC	
		Interassay %CV (norm response)	[redacted]% for HPC, MPC, LPC, and NC	
System suitability ranges	HPC ([redacted] ng/mL) MPC ([redacted] ng/mL) LPC ([redacted] ng/mL) NC (OD)	[redacted] ≤ HPC/NC ≤ [redacted] [redacted] ≤ MPC/NC ≤ [redacted] [redacted] ≤ LPC/NC ≤ [redacted] OD NC ≤ [redacted]		[redacted]325305QB20, Table 3, Table 4, Table 5, and Table 6
Drug tolerance	Mean drug tolerance at [redacted] ng/mL of PC: [redacted] µg/mL in screening and near [redacted] µg/mL in confirmatory assay. Mean drug tolerance at [redacted] ng/mL of PC: [redacted] µg/mL in screening and confirmatory assays.			[redacted]325305QB20, Table 8
Qualification of human serum pool	All acceptance criteria were met.			[redacted]325305QB20, Table 10
Hook effect	No hook effect was observed up to [redacted] ng/mL positive control.			[redacted]325305QB20, Table 7
Method performance in study ARGX-113-1802 (Module 5.3.1.4, [redacted]325305QB21**)**				
Assay passing rate	Screening assay performance: [redacted] runs accepted ([redacted]%) Confirmatory assay performance: [redacted] runs accepted ([redacted]%) Titration assay performance: [redacted] runs accepted ([redacted]%)			[redacted]235305QB21, Section 7.4
CPF in diseased population	The CP suitability assessment of the validation CP (CPF [redacted]) for the CIDP study population was verified by assessing the FPR in baseline samples. The validation CPF of [redacted] was suitable for use in the CIDP study population.			[redacted]325305QB21, Section 12.1
Selectivity	In participants with CIDP: [redacted] ([redacted]%) individuals scored positive at the LPC level of [redacted] ng/mL in screening and confirmatory assay.			[redacted]325305QB21, Section 12.8.1

2D11hFc=chimeric molecule consisting of efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone 2D11 and a human Fc; ACE=affinity capture elution; ADA=antidrug antibody(ies); CCP=confirmatory cut point; CIDP=chronic inflammatory demyelinating polyneuropathy; CP=cut point; CPF=cut point factor; CV=coefficient of variation; ECL=electrochemiluminescence; ELISA=enzyme-linked immunosorbent assay; EPT=endpoint titer; Fc=crystallizable fragment; FPR=false positive rate; HPC=high positive control; ITP=immune thrombocytopenia; LPC=low positive control; MPC=medium positive control; MRD=minimum required dilution; [redacted]; NC=negative control; no.=number; OD=optical density; PC=positive control; QC=quality control; SCP=screening cut-point

a) Supplemental validation of [redacted]314331QB10 is required following the introduction of new labeled materials.

表 2.7.1-10　エフガルチギモドに対する ADA 検出のための分析法パフォーマンスの要約－バリデーション Method #5

Bioanalytical method validation report name	**Validation of an Immunoassay for Screening, Confirmation, and Titration of Anti-ARGX-113 Antibodies in Human Serum Using ELISA (Module** 5.3.1.4, 8471-440**)**			
Method description	ADA against efgartigimod were detected with an ACE bridging ELISA.			
Material used for QCs	PC antibody: 2D11hFc Batch/lot no.: [redacted] Concentration: [redacted] mg/mL			
Source and lot of reagents	Efgartigimod, batch/lot no.: [redacted]			
MRD	Samples are tested undiluted (MRD=1)			
Validation parameters	**Method validation summary**			**Source location**
SCP factor determination	A multiplicative CPF of [redacted] was determined with sera from healthy Chinese individuals, ensuring a [redacted]% false positive error rate. The same CPF was used for the ADA titration assay.			8471-440, Table 2 and Section 16.5
CCP determination	CCP of [redacted]% inhibition was determined with sera from healthy Chinese participants, ensuring a [redacted]% false positive error rate. The drug concentration in the confirmatory tier was [redacted] µg/mL efgartigimod spiked in the master mix.			8471-440, Table 2 and Section 16.5
Sensitivity	Screening assay: [redacted] ng/mL Confirmatory assay: [redacted] ng/mL			8471-440, Table 4
Titer precision	MSR: [redacted] Endpoint titers were within [redacted] dilution step from the median titer			8471-440, Table 5 and Table 6
QC concentrations	HPC: [redacted] ng/mL MPC: [redacted] ng/mL LPC: [redacted] ng/mL NC: [redacted] ng/mL			8471-440, Table 9 and Table 10
Precision of QCs	**Screening assay**	**Intraassay %CV (S/NC values)**	[redacted]% for HPC, MPC, LPC, and NC (OD)	
		Interassay %CV (S/NC values)	[redacted]% for MPC and LPC [redacted]% for HPC and [redacted]% for NC (OD)	
		Interassay %CV (OD values)	[redacted]% for NC	
	Confirmatory assay	**Intraassay %CV (% inhibition)**	[redacted]% for HPC, MPC, and LPC	
		Interassay %CV (% inhibition)	[redacted]% for HPC, MPC, and LPC	
System suitability ranges	NC (OD) LPC (100 ng/mL) HPC (1000 ng/mL)	OD NC ≤ [redacted] [redacted] (NF) ≤ S/NC LPC ≤ [redacted] ≤ S/NC HPC ≤ [redacted]		8471-440, Table 12 and Table 13
Drug tolerance	**PC**	[redacted] ng/mL PC tolerated at least [redacted] µg/mL drug in the screening and confirmatory assays [redacted] ng/mL PC tolerated [redacted] µg/mL (mean, n=[redacted]) drug in the screening assay and at least [redacted] µg/mL in the confirmatory assay [redacted] ng/mL PC tolerated [redacted] µg/mL		8471-440, Table 19, Table 20, Table 21, Table 22, Table 23, and Table 24

Bioanalytical method validation report name	**Validation of an Immunoassay for Screening, Confirmation, and Titration of Anti-ARGX-113 Antibodies in Human Serum Using ELISA (Module** 5.3.1.4, 8471-440)		
		(mean, n=[redacted]) drug in the screening assay and [redacted] µg/mL (mean, n=[redacted]) in the confirmatory assay	
	PC2	[redacted] ng/mL PC tolerated at least [redacted]0 µg/mL drug in the screening and confirmatory assays [redacted] ng/mL PC tolerated [redacted] µg/mL drug in the screening assay and [redacted] µg/mL in the confirmatory assay [redacted] ng/mL PC tolerated [redacted] µg/mL drug in the screening assay and [redacted] µg/mL in the confirmatory assay	
Hook effect	No hook effect was observed up to [redacted] µg/mL of the PC.		8471-440, Table 18
Selectivity	No matrix effect in Chinese and non-Chinese healthy human sera.		8471-440, Table 14 and Table 15
Hemolysis effect	No matrix effect in hemolyzed serum.		8471-440, Table 16
Lipemic effect	No matrix effect in lipemic serum.		8471-440, Table 17
Bench-top/process stability	Up to [redacted] hours [redacted] minutes.		8471-440, Table 28
Refrigerator stability	Up to [redacted] hours [redacted] minutes.		8471-440, Table 29
Freeze-thaw stability	Up to [redacted] cycles at [redacted] °C to [redacted] °C and [redacted] °C to [redacted] °C.		8471-440, Table 26 and Table 27
Long-term storage	Up to [redacted] days at [redacted] °C to [redacted] °C and [redacted] °C to [redacted] °C.		8471-440, Table 30 and Table 31
Method performance in study ARGX-113-1802 (Module 5.3.1.4, 8478-707)			
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate)		8478-707, Section 15.1
CPF in diseased population	The CP suitability assessment of the validation CP (CPF [redacted]) for the CIDP study population was verified by assessing the FPR in baseline samples. The validation CPF of [redacted] was suitable for use in the CIDP study population.		8478-707, Section 15.3

2D11hFc=chimeric molecule consisting of efgartigimod-specific binding variable domain of a llama heavy chain only antibody clone 2D11 and a human Fc; ADA=antidrug antibody(ies); ARGX-113=efgartigimod; CCP=confirmatory cut point; CIDP=chronic inflammatory demyelinating polyneuropathy; CV=coefficient of variation; CP=cut point; CPF=cut point factor; FPR=false positive rate; HPC=high positive control; LPC=low positive control; MPC=mid positive control; MRD=minimum required dilution; MSR=minimum significant ratio; n=number of samples; NC=negative control; NF=normalization factor; OD=optical density; PC=positive control; QC=quality control; SCP=screening cut point; S/NC=signal to negative control

表 2.7.1-11　エフガルチギモドに対する ADA 検出のための分析法パフォーマンスの要約－Method #3 及び Method #5 のクロスバリデーション（[redacted] 社 対 [redacted]）

Bioanalytical method validation report name	**ARGX-113 ADA Method Cross-Validation** **(Module** 5.3.1.4, [redacted]353102QB10) **Validation of an Immunoassay for Screening, Confirmation, and Titration of Anti-ARGX-113 Antibodies in Human Serum Using ELISA (Addendum)** **(Module** 5.3.1.4, 8471-440) **Cross-Comparison of the Immunoassay for Screening, Confirmation, and Titration of Anti-ARGX-113 Antibodies in Human Serum Between [redacted][a] and [redacted]** **(Module** 5.3.1.4, ARGX-PTBA-1001)	
Method cross-comparison	Cross-comparison of the same ACE bridging ELISA at [redacted][a] ([redacted]353102QB10) and [redacted] (8471-440) using spiked PC samples.	
Parameters	**Cross-comparison performance**	**Source location**
Screening and confirmatory results	At both labs, all NC samples screened negative, and all PC samples (ie, LPC1 [redacted] ng/mL], LPC2 [redacted] ng/mL], MPC [redacted] ng/mL], and HPC [redacted] ng/mL]), spiked in normal human serum pool, screened and confirmed positive.	[redacted]353102QB10, Table 5 8471-440, Table 4 ARGX-PTBA-1001, Table 1 and Table 2
Titration results	Comparison of the EPT determined at both labs showed that the EPTs were within [redacted] titration step of each other at both spiked PC levels (ie, [redacted] ng/mL and [redacted] ng/mL PC).	[redacted]353102QB10, Table 9 8471-440, Table 5 ARGX-PTBA-1001, Table 3

ACE=affinity capture elution; ADA=antidrug antibody(ies); ARGX-113=efgartigimod; ELISA=enzyme-linked immunosorbent assay; EPT=endpoint titer; HPC=high positive control; LPC=low positive control; MPC=mid positive control; NAb=neutralizing antibody(ies); NC=negative control; PC=positive control
a) [redacted] was formerly known as [redacted] or [redacted].

表 2.7.1-12　エフガルチギモドに対する中和抗体検出のための分析法ライフサイクル

	Method validation #2	Clinical study ARGX-113-1802	Method validation #3	Clinical study ARGX-113-1802[a]
Analyte	NAb against efgartigimod			
Validation type	Validation; supplemental validation	In-study	Validation	In-study
eCTD reference number	[redacted]329686QB10 349215QB10	[redacted]338337QB20	8471-439	8478-707
Method ID	[redacted] version [redacted] ([redacted]329686QB10) [redacted] version [redacted] ([redacted]349215QB10)	[redacted] version [redacted]	[redacted]	[redacted]
Duration of time method is in use	[redacted] 20[redacted] to present ([redacted]329686QB10) [redacted] 20[redacted] to present ([redacted]349215QB10)	[redacted] 20[redacted] to [redacted] 20[redacted]	[redacted] 20[redacted] to present	[redacted] 20[redacted] to [redacted] 20[redacted]
Bioanalytical site	[redacted][b], [redacted], [redacted], [redacted], [redacted], UK		[redacted]	
Matrix	Human serum			
Platform	ECLIA			
Format	The NAb assay against efgartigimod is an indirect competition assay of efgartigimod and recombinant wild-type human IgG3 to human FcRn, where ADA are extracted using the BEAD procedure.		The NAb assay against efgartigimod is an indirect competition assay where ADA are extracted using the BEAD procedure.	

	Method validation #2	Clinical study ARGX-113-1802	Method validation #3	Clinical study ARGX-113-1802[a]
Stock reference, lot number, expiration date	**Positive control:** polyclonal rabbit anti-ARGX-113 Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted] **Positive control 2:** polyclonal rabbit anti-ARGX-113 Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted]	**Positive control:** polyclonal rabbit anti-ARGX-113 Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted]	**Positive control:** polyclonal rabbit anti-ARGX-113 Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]	**Positive control:** polyclonal rabbit anti-ARGX-113 Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]
Matrix study population	Healthy, CIDP, ITP, gMG, PV	CIDP	Healthy, Chinese	CIDP
Link to reports and applicable amendments	Module 5.3.1.4, [redacted]329686QB10, [redacted]349215QB10	Module 5.3.1.4, [redacted]338337QB20	Module 5.3.4.1, 8471-439	Module 5.3.1.4, 8478-707
Synopsis of amendment history	Bridging of efgartigimod batches [redacted] (old batch) and [redacted] (new batch); new batch is qualified for use	NA	NA	NA

ADA=antidrug antibody(ies); ARGX-113=efgartigimod; BEAD=biotin-drug extraction and acid dissociation; CIDP=chronic inflammatory demyelinating neuropathy; ECLIA=electrochemiluminescence immunoassay; eCTD=electronic common technical document; FcRn=neonatal Fc receptor; gMG=generalized myasthenia gravis; ID=identification; IgG=immunoglobulin γ; ITP=immune thrombocytopenia; NAb=neutralizing antibody(ies); no.=number; PV=pemphigus vulgaris; UK=United Kingdom

a) This method was used to analyze samples from participants in mainland China.

b) [redacted] was formerly known as [redacted] or [redacted].

表 2.7.1-13　エフガルチギモドに対する中和抗体検出のための分析法パフォーマンスの要約ーバリデーション Method #2

Bioanalytical method validation report name	**Validation of an Immunoassay for the Determination of Neutralizing Anti-ARGX-113 Antibodies in Human Serum (Module** 5.3.1.4, [redacted]329686QB10) **Supplemental Method Validation for Selectivity Assessment in Japanese Sera Within the ARGX-113 ADA (ELISA) and NAb ([redacted] ECL) Methods (Module** 5.3.1.4, [redacted]349215QB10)	
Method description	The NAb assay is an indirect ECLIA competition assay in which ADA are extracted using the BEAD procedure.	
Material used QCs	Polyclonal rabbit anti-ARGX-113, batch/lot no.: [redacted]	
Source and lot of reagents	Efgartigimod, batch/lot no.: [redacted] and batch/lot no.: [redacted], argenx	
MRD	Samples are tested undiluted (MRD=1).	
Validation parameters	**Method validation summary**	**Source location**
CPF determination	A multiplicative CPF of [redacted] was determined with [redacted] sera from healthy participants to ensure a [redacted]% false positive error rate.	[redacted]329686QB10, Table 18 and Appendix 5
Hook effect	No hook effect was observed up to [redacted] µg/mL PC.	[redacted]329686QB10, Table 8 and Figure 1
Assay sensitivity	Relative assay sensitivity was calculated at [redacted] µg/mL PC at [redacted]% CI.	[redacted]329686QB10, Table 2 and Appendix 6
Selectivity	**Healthy individuals:** [redacted] ([redacted]%) individuals scored positive at LPC1 and LPC2 levels of [redacted] µg/mL and [redacted] µg/mL, respectively. **Participants with ITP:** [redacted] ([redacted]%) individuals scored positive at LPC1 and LPC2 levels of [redacted] µg/mL and [redacted] µg/mL, respectively. **Participants with gMG:** [redacted] ([redacted]%) individuals scored positive at LPC1 and LPC2 levels of [redacted] µg/mL and [redacted] µg/mL, respectively. **Participants with CIDP:** [redacted] ([redacted]%) individuals scored positive at LPC1 and LPC2 levels of [redacted] µg/mL and [redacted] µg/mL, respectively. **Participants with PV:** [redacted] ([redacted]%) individuals scored positive at LPC1 and LPC2 levels of [redacted] µg/mL and [redacted] µg/mL, respectively. **Healthy Japanese individuals:** [redacted] ([redacted]%) individuals scored positive at LPC level of [redacted] µg/mL.	[redacted]329686QB10, Table 9 [redacted]349215QB10, Section 9.2
Drug tolerance	[redacted] µg/mL PC is detectable in presence of [redacted] µg/mL efgartigimod. [redacted] µg/mL PC is detectable in presence of [redacted] µg/mL efgartigimod. [redacted] µg/mL PC is detectable in presence of [redacted] µg/mL efgartigimod. [redacted] µg/mL PC is detectable in presence of [redacted] µg/mL efgartigimod. [redacted] µg/mL PC is detectable in presence of [redacted] µg/mL efgartigimod.	[redacted]329686QB10, Table 11 and Table 12

Bioanalytical method validation report name	**Validation of an Immunoassay for the Determination of Neutralizing Anti-ARGX-113 Antibodies in Human Serum (Module** 5.3.1.4, [redacted]329686QB10**)** **Supplemental Method Validation for Selectivity Assessment in Japanese Sera Within the ARGX-113 ADA (ELISA) and NAb ([redacted] ECL) Methods (Module** 5.3.1.4, [redacted]349215QB10**)**			
QC concentrations	HPC: [redacted] µg/mL MPC: [redacted] µg/mL LPC1: [redacted] µg/mL LPC2: [redacted] µg/mL NC: [redacted] µg/mL			[redacted]329686QB10, Section 11.6
Precision of QC	**Screening assay**	**Intraassay %CV (response)**	[redacted]% for HPC, MPC, LPC1, and LPC2 [redacted]% for NC (anomalous NC response, mean CV% is [redacted])	
		Interassay %CV (norm response)	[redacted]% for HPC, MPC, LPC1, and LPC2	
System suitability ranges	NC (RLU)	RLU NC ≤ [redacted]		[redacted]329686QB10, Table 7
	LPC1 ([redacted] µg/mL)	[redacted] ≤ LPC1/NC ≤ [redacted]		
	LPC2 ([redacted] µg/mL)	[redacted] ≤ LPC2/NC ≤ [redacted]		
	HPC ([redacted] µg/mL)	[redacted] ≤ HPC/NC ≤ [redacted]		
Hemolysis effect	LPC1 and LPC2 scores positive in [redacted] ([redacted]%) hemolytic samples			[redacted]329686QB10, Table 10
Lipemic effect	LPC1 and LPC2 scores positive in [redacted] ([redacted]%) lipemic samples			[redacted]329686QB10, Table 10
Bench-top/process stability	Up to [redacted] hours with samples initially stored at [redacted] °C ± [redacted] °C.			[redacted]329686QB10, Table 13
Freeze-thaw stability	Up to [redacted] freeze-thaw cycles with samples initially stored at [redacted] °C ± [redacted] °C.			[redacted]329686QB10, Table 13
Long-term storage	Up to [redacted] days at [redacted] °C ± [redacted] °C.			[redacted]329686QB10, Table 13
Method performance in study ARGX-113-1802 (Module 5.3.1.4, [redacted]338337QB20**)**				
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).			[redacted]338337QB20, Section 7.4
CP suitability assessment	The CP suitability assessment of the validation CP (CPF [redacted]) for the CIDP study population was verified by assessing the FPR in screening samples by comparing the means and variances in CIDP and validation population and recalculating the CPF. The validation CPF of [redacted] was suitable for use in the CIDP study population.			[redacted]338337QB20, Section 11.3

ADA=antidrug antibodies; ARGX-113=efgartigimod; BEAD=biotin-drug extraction and acid dissociation; CIDP=chronic inflammatory demyelinating polyneuropathy; CP=cut point; CPF=cut point factor; CV=coefficient of variation; ECL=electrochemiluminescence; ECLIA=electrochemiluminescence immunoassay; ELISA=enzyme-linked immunosorbent assay; FPR=false positive rate; gMG=generalized myasthenia gravis; HPC=high positive control; ITP=immune thrombocytopenia; LPC=low positive control; MPC=medium positive control; MRD=minimum required dilution; [redacted]; NA=not applicable; NAb=neutralizing antibody(ies); NC=negative control; no.=number; PC=positive control; PV=pemphigus vulgaris; QC=quality control; RLU=relative light units

表 2.7.1-14　エフガルチギモドに対する中和抗体検出のための分析法パフォーマンスの要約ーバリデーション Method #3

Bioanalytical method validation report name	**Validation of an Immunoassay for Determination of Neutralizing Antibodies against ARGX-113 in Human Serum Using ECLIA (Module** 5.3.4.1, 8471-439**)**		
Method description	The NAb assay is an indirect ECLIA competition assay in which ADA are extracted using the BEAD procedure.		
Material used QCs	Polyclonal rabbit anti-ARGX-113, batch/lot no.: [redacted]		
Source and lot of reagents	Efgartigimod, batch/lot no: [redacted], argenx		
MRD	Samples are tested undiluted (MRD=1).		
Validation parameters	**Method validation summary**		**Source location**
CPF determination	A multiplicative CPF of [redacted] was determined with [redacted] sera from healthy Chinese individuals, ensuring a [redacted]% false positive error rate.		8471-439, Table 2
Hook effect	No hook effect was observed up to [redacted] µg/mL positive control.		8471-439, Table 10
Assay sensitivity	The assay sensitivity was determined as [redacted] µg/mL in the assay at [redacted]%CI		8471-439, Table 2
Sensitivity confirmation (selectivity testing)	No matrix effect in Chinese normal human serum.		8471-439, Table 7
Drug tolerance	[redacted] µg/mL PC is detectable in presence of at least [redacted] µg/mL (mean and median) efgartigimod. [redacted] µg/mL PC is detectable in presence of [redacted] µg/mL (mean) and [redacted] µg/mL (median) efgartigimod. [redacted] µg/mL PC is detectable in presence of [redacted] µg/mL (mean) and [redacted] µg/mL (median) efgartigimod.		8471-439, Table 11
QC concentrations	HPC: [redacted] µg/mL MPC: [redacted] µg/mL LPC: [redacted] µg/mL NC: [redacted] µg/mL		8471-439, Table 3 and Table 4
Precision of QCs	**Screening assay**	**Intraassay %CV (S/NC values)**	[redacted]% for HPC % for MPC % for LPC
		Intraassay %CV (response)	[redacted]% for NC
		Interassay %CV (S/NC values)	[redacted]% for HPC % for MPC % for LPC
		Interassay %CV (response)	[redacted]% for NC
System suitability ranges	NC (RLU) LPC ([redacted] µg/mL) HPC ([redacted] µg/mL)	RLU NC ≤ [redacted] [redacted] ≤ S/N LPC ≤ [redacted] [redacted] ≤ S/N HPC	8471-439, Table 5 and Table 6
Hemolysis effect	No matrix effect in hemolyzed serum.		8471-439, Table 8

Bioanalytical method validation report name	**Validation of an Immunoassay for Determination of Neutralizing Antibodies against ARGX-113 in Human Serum Using ECLIA (Module** 5.3.4.1, 8471-439**)**	
Lipemic effect	No matrix effect in lipemic serum.	8471-439, Table 9
Bench-top/process stability	Up to [redacted] hours [redacted] minutes.	8471-439, Table 13
Refrigerator stability	Up to [redacted] hours.	8471-439, Table 14
Freeze-thaw stability	Up to [redacted] freeze-thaw cycles at [redacted] °C to [redacted] °C and [redacted] °C to [redacted] °C.	8471-439, Table 12
Long-term storage	Up to [redacted] days at [redacted] °C to [redacted] °C and [redacted] °C to [redacted] °C.	8471-439, Table 15
Method performance in study ARGX-113-1802 (Module 5.3.1.4, 8478-707**)**		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	8478-707, Section 16.1

ADA=antidrug antibodies; ARGX-113=efgartigimod; BEAD=biotin-drug extraction and acid dissociation; CP=cut point; CPF=cut point factor; CV=coefficient of variation; ECLIA=electrochemiluminescence immunoassay; HPC=high positive control; LPC=low positive control; MPC=medium positive control; MRD=minimum required dilution; NAb=neutralizing antibody(ies); NC=negative control; no.=number; PC=positive control; QC=quality control; RLU=relative light units; S/N=signal to noise

表 2.7.1-15　rHuPH20 に対する抗体検出のための分析法ライフサイクル

	Validation method #1	**Validation method #1 addendum 3**	**Validation method #1 addendum 5**	**Clinical study ARGX-113-1802**	**Validation method #2**	**Clinical study ARGX-113-1802[a]**	**Cross-validation methods #1 and #2**
Analyte	Antibodies against rHuPH20 (hyaluronidase)						
Validation type	Validation; supplemental validation	Validation Addendum 3	Validation Addendum 5	In-study	Validation	In-study	Cross-validation
eCTD reference number	HALO 20079 rev2, HALO 21111 rev1	HALO 22076 rev0	HALO 23045 rev1	8442-925	HALO 21040 rev0	8486-448	HALO 22029 rev0
Method ID	[redacted]			[redacted]	[redacted]	[redacted]-V[redacted]	[redacted]: [redacted]:
Duration of time method is in use	[redacted] 20[redacted] to present	[redacted] 20[redacted] to present	[redacted] 20[redacted] to present	[redacted] 20[redacted] to 20[redacted]	[redacted] 20[redacted] to present	[redacted] 20[redacted] to 20[redacted]	[redacted] 20[redacted] to present
Bioanalytical site	[redacted][b], [redacted], [redacted], [redacted], US				[redacted]		[redacted][b], [redacted], US

	Validation method #1	Validation method #1 addendum 3	Validation method #1 addendum 5	Clinical study ARGX-113-1802	Validation method #2	Clinical study ARGX-113-1802[a]	Cross-validation methods #1 and #2
Matrix	Human plasma ([redacted])						
Platform	[redacted] platform						
Format	Antibodies against rHuPH20 in human plasma are detected by applying a bridging format using biotinylated rHuPH20 as capture and [redacted]-labeled rHuPH20 as detection tool.						
Stock reference Lot number Expiration date	**rHuPH20:** Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted] Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-PH20 antibody Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted]	**rHuPH20:** Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-PH20 antibody Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted]	**rHuPH20:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-PH20 antibody Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted] Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]	**rHuPH20:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-PH20 antibody Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted]	**rHuPH20:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-rHuPH20 IgG fraction (PC) Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] Rabbit polyclonal anti-rHuPH20 affinity purified antibody (PC2) Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]	**rHuPH20:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-PH20 antibody Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]	**rHuPH20:** [redacted] *and* Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-rHuPH20 IgG fraction (PC) [redacted] Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted] Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]
Matrix study population	Healthy, CIDP, MG, PV	Healthy	Healthy	CIDP	Healthy, Chinese	CIDP	Healthy
Link to reports and applicable amendments	gMG SC CTD, Module 5.3.1.4, HALO 20079 rev2, HALO 21111	Module 5.3.1.4, HALO 22076 rev0	Module 5.3.1.4, HALO 23045 rev1	Module 5.3.1.4, 8442-925	Module 5.3.1.4, HALO 21040 rev0	Module 5.3.1.4, 8486-448	Module 5.3.1.4, HALO 22029 rev0

	Validation method #1	Validation method #1 addendum 3	Validation method #1 addendum 5	Clinical study ARGX-113-1802	Validation method #2	Clinical study ARGX-113-1802[a]	Cross-validation methods #1 and #2
	rev1						
Synopsis of amendment history	NA	NA	NA	NA	NA	NA	NA

CIDP=chronic inflammatory demyelinating polyneuropathy; ECL=electrochemiluminescence; eCTD=electronic common technical document; ID=identification; IgG=immunoglobulin γ; [redacted]=[redacted]; MG=myasthenia gravis; [redacted]=[redacted]; NA=not applicable; no.=number; PC=positive control; PV=pemphigus vulgaris; rev=revision; rHuPH20=recombinant human hyaluronidase; SH=[redacted]; US=United States

a) This method was used to analyze samples from participants in mainland China.

b) Formerly known as [redacted].

表 2.7.1-16　rHuPH20 に対する抗体検出のための分析法パフォーマンスの要約－バリデーション Method #1 及び補足バリデーション Method #1

Bioanalytical method validation report name	**Validation of an Immunoassay for Detection, Confirmation, and Titration of Anti-rHuPH20 Antibodies in Human Plasma ([redacted])** **(gMG SC CTD, Module 5.3.1.4, HALO 20079 rev2)** **Selectivity Assessment in CIDP, MG, and PV Disease Human Plasma and Drug Interference Evaluation for Efgartigimod in the Validated Method for the Detection of Anti-rHuPH20 Antibodies in Human [redacted] Plasma** **(gMG SC CTD, Module 5.3.1.4, HALO 21111 rev1)**	
Method description	Antibodies against recombinant human rHuPH20 (hyaluronidase) are detected with a bridging ADA assay on the [redacted] platform. Method validation details are included in the gMG SC CTD, Module 2.7.1, Table 23.	
Validation parameters	**Method validation summary**	**Source location**
Selectivity	**In participants with MG:** [redacted] ([redacted]%) individuals confirmed positive at an LPC level of [redacted] ng/mL and an HPC level of [redacted] ng/mL. **In participants with CIDP:** [redacted] ([redacted]%) individuals confirmed positive at LPC of [redacted] ng/mL and HPC level of [redacted] ng/mL. **In participants with PV:** [redacted] ([redacted]%) individuals confirmed positive at LPC of [redacted] ng/mL and HPC level of [redacted] ng/mL.	HALO 21111 rev1, Table 5 HALO 21111 rev1, Table 4 HALO 21111 rev1, Table 6
Method performance in study ARGX-113-1802 (Module 5.3.1.4, 8442-925**)**		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	8442-925, Table 1
SCP factor in diseased population	The representativeness of the validation SCP (CPF [redacted]) for the CIDP study population was verified by assessing the FPR in screening samples. The validation CPF of [redacted] was suitable for use in the CIDP study population.	8442-925, Section 4.4

ADA=antidrug antibody(ies); ARGX-113=efgartigimod; CIDP=chronic inflammatory demyelinating polyneuropathy; CPF=cut point factor; CV=coefficient of variation; FPR=false positive rate; gMG=generalized myasthenia gravis; HPC=high positive control; LPC=low positive control; MG=myasthenia gravis; [redacted]=[redacted]; NC=negative control; PC=positive control; PSCP=plate-specific cut point; PV=pemphigus vulgaris; rev=revision; rHuPH20=recombinant human hyaluronidase; RLU=relative light units; SCP=screening cut point

表 2.7.1-17　rHuPH20 に対する抗体検出のための分析法パフォーマンスの要約－バリデーション Method #1 補遺 3

Bioanalytical method validation report name	**Validation of an Immunoassay for Detection, Confirmation, and Titration of Anti-rHuPH20 Antibodies in Human Plasma ([redacted]) Report Addendum 3 (Module** 5.3.1.4, HALO 22076 rev0**)**	
Method description	Antibodies against recombinant human rHuPH20 (hyaluronidase) were detected with a bridging ADA assay on the [redacted] platform.	
Material used for QCs	PC antibody: Rabbit polyclonal anti-PH20 antibody Batch/lot no.: [redacted] Concentration: [redacted] mg/mL	
Source and lot of reagents	rHuPH20, batch/lot no.: [redacted]	
MRD	1/[redacted]	
Validation parameters	**Method validation summary**	**Source location**
QC concentrations	HPC: [redacted] ng/mL LPC: [redacted] ng/mL NC: [redacted] ng/mL	HALO 22076 rev0, Table 2
System suitability criteria	HPC ([redacted] ng/mL) ≤ HPC ≤ [redacted] LPC ([redacted] ng/mL) [redacted] ≤ LPC ≤ [redacted] NC ([redacted] ng/mL) [redacted] ≤ NC ≤ [redacted]	

ADA=antidrug antibody(ies); HPC=high positive control; LPC=low positive control; [redacted]; NC=negative control; PC=positive control; QC=quality control; rev=revision; rHuPH20=recombinant human hyaluronidase PH20

表 2.7.1-18　rHuPH20 に対する抗体検出のための分析法パフォーマンスの要約－バリデーション Method #1 補遺 5

Bioanalytical method validation report name	**Validation of an Immunoassay for Detection, Confirmation, and Titration of Anti-rHuPH20 Antibodies in Human Plasma ([redacted]) Report Addendum No. 5** **(Module** 5.3.1.4, HALO 23045 rev1**)**	
Method description	Antibodies against recombinant human rHuPH20 (hyaluronidase) were detected with a bridging ADA assay on the [redacted] platform.	
Material used for QCs	PC antibody: Rabbit polyclonal anti-PH20 antibody Batch/lot no.: [redacted] Concentration: [redacted] mg/mL Batch/lot no.: [redacted] Concentration: [redacted] mg/mL	
Source and lot of reagents	rHuPH20, batch/lot no. [redacted]	
MRD	1/[redacted]	
Validation parameters	**Method validation summary**	**Source location**
Bridging new PC	Using method [redacted], lot [redacted] at concentrations of [redacted] and [redacted] ng/mL (LPC and HPC, respectively) exhibited response values within the system suitability ranges set by lot [redacted] at concentrations of [redacted] and [redacted] ng/mL (LPC and HPC, respectively). Lot [redacted] was successfully bridged with lot [redacted] in the method [redacted].	HALO 23045 rev1, Table 2
QC concentrations	HPC: [redacted] ng/mL LPC: [redacted] ng/mL NC: [redacted] ng/mL	HALO 23045 rev1, Table 2
Long-term storage	[redacted] days at [redacted] °C or [redacted] °C to [redacted] °C	HALO 23045 rev1, Page 10

ADA=antidrug antibody(ies); HPC=high positive control; LPC=low positive control; [redacted]=[redacted]; NC=negative control; PC=positive control; QC=quality control; rev=revision; rHuPH20=recombinant human hyaluronidase PH20

表 2.7.1-19　rHuPH20 に対する抗体検出のための分析法パフォーマンスの要約－バリデーション Method #2

Bioanalytical method validation report name	**Validation of an Immunoassay for Detection, Confirmation, and Titration of Anti-rHuPH20 Antibodies in Human Plasma ([redacted])** **(Module** 5.3.1.4, HALO 21040 rev0**)**		
Method description	Antibodies against rHuPH20 (hyaluronidase) are detected with a bridging ADA assay on the [redacted] platform.		
Material used for QCs	PC: rabbit polyclonal anti-PH20 antibody Batch/lot no. [redacted] Concentration: [redacted] mg/mL PC2: rabbit polyclonal anti-rHuPH20 affinity purified antibody Batch/lot no.: [redacted] Concentration: [redacted] mg/mL		
Source and lot of reagents	rHuPH20, batch/lot no.: [redacted]		
MRD	1/[redacted]		
Validation parameters	**Method validation summary**		**Source location**
SCP factor determination	The multiplicative screening CPF of [redacted] was determined with plasma from [redacted] healthy participants (White), ensuring a [redacted]% FPR with [redacted]% LCL.		HALO 21040 rev0, Table 2 and Annex 14.5
CCP determination	CCP of [redacted]% inhibition was determined with plasma from [redacted] healthy participants (White), ensuring a [redacted]% FPR with [redacted]% LCL.		HALO 21040 rev0, Table 2 and Annex 14.5
Titer CPF determination	The multiplicative screening CPF of [redacted] was determined with plasma from [redacted] healthy participants (White), ensuring a [redacted]% FPR.		HALO 21040 rev0, Table 2 and Annex 14.5
Sensitivity	PC	Screening assay sensitivity: [redacted] ng/mL	HALO 21040 rev0, Table 4
		Confirmatory assay sensitivity: [redacted] ng/mL	
	PC2 (only used in LPC determination)	Screening assay sensitivity: [redacted] ng/mL.	HALO 21040 rev0, Table 3
		Confirmatory assay sensitivity: [redacted] ng/mL.	
Target titer	Median titer: [redacted]		HALO 21040 rev0, Table 8
Titer precision	All titer values are within [redacted] dilution step from the median titer		HALO 21040 rev0, Table 8
Precision of QCs	**QC concentrations**	HPC: [redacted] ng/mL MPC: [redacted] ng/mL LPC: [redacted] ng/mL NC: [redacted] ng/mL	HALO 21040 rev0, Table 7

Bioanalytical method validation report name	**Validation of an Immunoassay for Detection, Confirmation, and Titration of Anti-rHuPH20 Antibodies in Human Plasma ([redacted])** (**Module** 5.3.1.4, HALO 21040 rev0)				
	Precision of QCs	**Screening assay**	**Intraassay %CV (response)**	[redacted]% for HPC, MPC, LPC, and NC	HALO 21040 rev0, Table 7
			Interassay %CV (response)	[redacted]% for HPC, MPC, LPC, and NC	
		Confirmatory assay	**Intraassay %CV (% inhibition)**	[redacted]% for HPC, MPC, and LPC	
			Interassay %CV (% inhibition)	[redacted]% for HPC, MPC, and LPC	
System suitability ranges	NC (RLU) LPC ([redacted] ng/mL) HPC ([redacted] ng/mL)	NC (RLU) ≤ [redacted] [redacted] ≤ LPC (RLU) ≤ [redacted] [redacted] ≤ HPC (RLU) ≤ [redacted]			HALO 21040 rev0, Table 5 and Table 6
Selectivity	No matrix effect in Chinese and non-Chinese normal matrix.				HALO 21040 rev0, Table 9 and Table 10
Hemolysis effect	No matrix effect in [redacted]% hemolyzed matrix.				HALO 21040 rev0, Table 11
Lipemic effect	No matrix effect in lipemic matrix ([redacted] mg/dL of triglycerides).				HALO 21040 rev0, Table 12
Interference	PC [redacted] ng/mL can tolerate [redacted] ng/mL of rHuPH20				HALO 21040 rev0, Table 13
Hook effect	No hook effect was observed up to a maximum concentration of [redacted] ng/mL of PC.				HALO 21040 rev0, Table 14
Bench-top/process stability	Up to [redacted] hours and [redacted] minutes.				HALO 21040 rev0, Table 16
Refrigerator stability	Up to [redacted] hours and [redacted] minutes.				HALO 21040 rev0, Table 17
Freeze-thaw stability	Up to [redacted] freeze-thaw cycles at [redacted] °C to [redacted] °C and [redacted] °C to [redacted] °C.				HALO 21040 rev0, Table 15
Long-term storage	Up to [redacted] days at [redacted] °C to [redacted] °C and [redacted] °C to [redacted] °C.				HALO 21040 rev0, Table 18

Bioanalytical method validation report name	**Validation of an Immunoassay for Detection, Confirmation, and Titration of Anti-rHuPH20 Antibodies in Human Plasma ([redacted])** **(Module** 5.3.1.4, HALO 21040 rev0)	
Method performance in study ARGX-113-1802 **(Module** 5.3.4.1, 8486-448)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	8486-448, Section 13.1
SCP factor in diseased population	The representativeness of the validation SCP (CPF [redacted]) for the CIDP study population was verified by assessing the FPR in baseline samples and by comparing the means and variances in CIDP and validation population. The validation CPF of [redacted] was suitable for use in the CIDP study population.	8486-448, Section 13.3

ADA=antidrug antibody(ies); CCP=confirmatory cut point; CIDP=chronic inflammatory demyelinating polyneuropathy; CPF=cut point factor; CV=coefficient of variation; FPR=false positive rate; HPC=high positive control; LCL=lower confidence limit; LPC=low positive control; [redacted]=[redacted]; NC=negative control; PC=positive control; QC=quality control; rev=revision; rHuPH20=recombinant human hyaluronidase PH20; RLU=relative light units

表 2.7.1-20　rHuPH20 に対する抗体検出のための分析法パフォーマンスの要約－Method #1 及び Method #2 のクロスバリデーション

Bioanalytical method validation report name	**Cross-Validation of an ECLIA for Detection, Confirmation, and Titration of Anti-rHuPH20 Antibodies in Human Plasma ([redacted]) (Module** 5.3.1.4, HALO 22029 rev0)	
Method cross-validation	Cross-validation of the same bridging ECL method at [redacted] and [redacted] using spiked matrix and blank matrix samples.	
Parameters	**Cross-validation performance**	**Source location**
Cross-validation results	[redacted] ([redacted]%) samples spiked with PC ranging from [redacted] to [redacted] ng/mL (LPC and HPC, respectively), and blank sample, passed the acceptance criteria for cross-validation samples between the [redacted] and [redacted] laboratories. Unspiked samples screened or confirmed negative at both the [redacted] and [redacted] laboratories. All spiked samples screened and confirmed positive, including those additionally spiked with rHuPH20. All spiked samples yielded titer values within [redacted]-fold dilution of each other.	HALO 22029 rev0, Table 3

ECLIA=electrochemiluminescence immunoassay; HPC=high positive control; LPC=low positive control; PC=positive control; rev=revision; rHuPH20=recombinant human hyaluronidase PH20; SH=[redacted]; US=United States

表 2.7.1-21　rHuPH20 に対する中和抗体検出のための分析法ライフサイクル

	Method validation #1, supplemental validation	**Clinical study ARGX-113-1802**	**Method validation #2**	**Clinical study ARGX-113-1802[a]**	**Cross-validation methods #1 and #2**	**Method validation #1 – confirmatory tier**	**Method validation #2 – confirmatory tier**
Analyte	NAb against rHuPH20						
Validation type	Validation, supplemental validation	In-study	Validation	In-study	Cross-validation	Partial validation/ addition of a confirmatory tier	Partial validation/ addition of a confirmatory tier
eCTD reference number	HALO 20078 rev3, HALO 22047 rev0	8442-925, 8442-925 addendum01	HALO 21075 rev0	8486-448, 8486-448 amendment01	HALO 23115 rev0	HALO 24008 rev0, HALO 24009 rev0	HALO 24028 rev0
Method ID	[redacted]	[redacted]	[redacted]4	[redacted]- V [redacted]- V	[redacted]: [redacted]: [redacted]-V	[redacted]	[redacted]-V -V
Duration of time method is in use	[redacted] 20[redacted] to present	[redacted] 20[redacted] to 20[redacted]	[redacted] 20[redacted] to present	[redacted] 20[redacted] to 20[redacted]	[redacted] 20[redacted] to present	[redacted] 20[redacted] to present	[redacted] 20[redacted] to present
Bioanalytical site	[redacted][b], US	[redacted]	[redacted]	[redacted]	[redacted][b], US	[redacted][b], US	[redacted]

	Method validation #1, supplemental validation	Clinical study ARGX-113-1802	Method validation #2	Clinical study ARGX-113-1802[a]	Cross-validation methods #1 and #2	Method validation #1 – confirmatory tier	Method validation #2 – confirmatory tier
Matrix	Human plasma ([redacted])					Human plasma ([redacted]) Human plasma ([redacted])	Human plasma ([redacted])
Platform	ELISA						
Format	The NAb assay against rHuPH20 is an assay to detect NAb that interfere with rHuPH20 activity to digest HA (coated on ELISA plate). [redacted] is added to detect remaining undegraded HA on the surface. Streptavidin-HRP is used as a detection tool.					The NAb assay against rHuPH20 is an assay to detect NAb that interfere with rHuPH20 activity to digest HA (coated on ELISA plate). [redacted] is added to detect remaining undegraded HA on the surface. Streptavidin-HRP is used as detection tool. The confirmation assay is an immuno-depletion step based on the use of immunoglobulin-specific recombinant proteins conjugated to magnetic beads to deplete anti-rHuPH20 NAb.	

	Method validation #1, supplemental validation	**Clinical study ARGX-113-1802**	**Method validation #2**	**Clinical study ARGX-113-1802[a]**	**Cross-validation methods #1 and #2**	**Method validation #1 – confirmatory tier**	**Method validation #2 – confirmatory tier**
Stock reference, lot number, expiration date	**rHuPH20:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit anti-rHuPH20 IgG fraction Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted] Batch/lot no.: [redacted] Re-test date: [redacted] 20[redacted]	**rHuPH20:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-rHuPH20 antibody Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted]	**rHuPH20:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-rHuPH20 IgG fraction Batch/lot no: [redacted] Re-test date: [redacted] 20[redacted]	**rHuPH20:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-rHuPH20 IgG fraction Batch/lot no: [redacted] Re-test date: [redacted] 20[redacted]	**rHuPH20:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-rHuPH20 IgG fraction Batch/lot no: [redacted] Re-test date: [redacted] 20[redacted]	**rHuPH20:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-rHuPH20 IgG fraction Batch/lot no: [redacted] Re-test date: [redacted] 20[redacted]	**rHuPH20:** Batch/lot no.: [redacted] Exp. date: [redacted] 20[redacted] **Positive control:** Rabbit polyclonal anti-rHuPH20 IgG fraction Batch/lot no: [redacted] Re-test date: [redacted] 20[redacted]
Matrix study population	Healthy, CIDP, MG, PV	CIDP	Healthy, Chinese	CIDP	Healthy	Healthy, CIDP, MG, PV, ITP	Healthy, Chinese
Link to reports and applicable amendments	gMG SC CTD, Module 5.3.1.4, HALO 20078 rev3, HALO 22047 rev0	Module 5.3.1.4, 8442-925, 8442-925 addendum01	Module 5.3.1.4, HALO 21075 rev0	Module 5.3.1.4, 8486-448, 8486-448 amendment01	Module 5.3.1.4, HALO 23115 rev0	Module 5.3.1.4, HALO 24008 rev0, HALO 24009 rev0	Module 5.3.1.4, HALO 24028 rev0
Synopsis of amendment history	NA	NA	NA	NA	NA	NA	NA

CIDP=chronic inflammatory demyelinating neuropathy; ELISA=enzyme-linked immunosorbent assay; HA=hyaluronate; HRP=horse radish peroxidase; IgG=immunoglobulin γ; [redacted]=[redacted]; ITP=immune thrombocytopenia; MG=myasthenia gravis; NA=not applicable; NAb=neutralizing antibody(ies); no.=number; PV=pemphigus vulgaris; rev=revision; rHuPH20=recombinant human hyaluronidase PH20; SH=[redacted]; US=United States

a) This method was used to analyze samples from participants in mainland China.

b) [redacted] was formerly known as [redacted].

表 2.7.1-22　rHuPH20 に対する中和抗体検出のための分析法パフォーマンスの要約（スクリーニング及びタイトレーションアッセイ）－バリデーション Method #1 及び補足バリデーション Method #1

Bioanalytical method validation report name	**Validation of Enzyme Activity Assay for the Detection of rHuPH20-Neutralizing Activity in Human [redacted] Plasma (gMG SC CTD, Module 5.3.1.4, HALO 20078 rev3)** **Selectivity Assessment in CIDP, MG, and PV Disease Human Plasma and Drug Interference Evaluation for Efgartigimod in the Validated Method for the Detection of Neutralizing Antibodies to rHuPH20 in Human [redacted] Plasma (gMG SC CTD, Module 5.3.1.4, HALO 22047 rev0)**	
Method description	The NAb assay against rHuPH20 is an assay to detect NAb that interfere with rHuPH20 activity to digest HA (coated on ELISA plate). [redacted] is added to detect remaining undegraded HA on the surface. Streptavidin-HRP is used as detection tool. Method validation details are included in the gMG SC CTD, Module 2.7.1, Table 25.	
Selectivity	Healthy individuals: (PC lot #[redacted]) [redacted] ([redacted]%) individuals scored positive at LPC level of [redacted] µg/mL and at HPC level of [redacted] µg/mL. Patients with PV: (PC lot #[redacted]) [redacted] ([redacted]%) individuals scored positive at LPC level of [redacted] µg/mL and at an HPC level of [redacted] µg/mL. Patients with CIDP: (PC lot #[redacted]) [redacted] ([redacted]%) individuals scored positive at an LPC level of [redacted] µg/mL, and [redacted] ([redacted]%) individuals scored positive at an HPC level of [redacted] µg/mL. Patients with MG: (PC lot #[redacted]) [redacted] ([redacted]%) individuals scored positive at an LPC level of [redacted] µg/mL and at an HPC level of [redacted] µg/mL.	HALO 20078 rev3, Table 7A HALO 22047 rev0, Table 4 HALO 22047 rev0, Table 5 HALO 22047 rev0, Table 6
Method performance in ARGX-113-1802 (Module 5.3.1.4, 8442-925)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	8442-925, Section 5

CIDP=chronic inflammatory demyelinating neuropathy; ELISA=enzyme-linked immunosorbent assay; HA=hyaluronate; HPC=high positive control; HRP=horse radish peroxidase; IgG=immunoglobulin gamma; LPC=low positive control; MG=myasthenia gravis; NA=not applicable; NAb=neutralizing antibody(ies); no.=number; PC=positive control; PV=pemphigus vulgaris; rev=revision; rHuPH20=recombinant human hyaluronidase PH20

表 2.7.1-23　rHuPH20 に対する中和抗体検出のための分析法パフォーマンスの要約（スクリーニング及びタイトレーションアッセイ）－バリデーション Method #2

Bioanalytical method validation report name	**Validation for the Detection of Neutralizing Anti-rHuPH20 Antibody in Human [redacted] Plasma Using Hyaluronidase Activity Assay (Module** 5.3.1.4, HALO 21075 rev0**)**				
Method description	The NAb against rHuPH20 assay detects NAb that interfere with rHuPH20 activity to digest HA (coated on an ELISA plate). [redacted] is added to detect remaining undegraded HA on the surface. Streptavidin-HRP is used as a detection tool.				
Material used QCs	Rabbit polyclonal anti-rHuPH20 IgG fraction, batch/lot no: [redacted]				
Source and lot of reagents	rHuPH20, batch/lot no: [redacted]				
MRD	1/[redacted]				
Validation parameters	**Method validation summary**				**Source location**
SCP factor determination	The cut point NF was calculated as [redacted] with [redacted] healthy participants (Caucasian), ensuring a [redacted]% false positive error rate. PSCP=NEC/NF.				HALO 21075 rev0, Table 2
Titration CPF determination	The cut point NF was calculated as [redacted] with [redacted] healthy participants (Caucasian), ensuring a [redacted]% false positive error rate. PSCP=NEC/NF.				HALO 21075 rev0, Table 2
Sensitivity	The assay sensitivity was calculated as [redacted] µg/mL at [redacted]% CI.				HALO 21075 rev0, Table 4
Precision of QCs	**QC concentrations**	HPC: [redacted] µg/mL LPC: [redacted] µg/mL			HALO 21075 rev0, Table 5
	Precision of QCs	**Screening assay**	**Intraassay %CV (response)**	[redacted]% for HPC, LPC, and NEC [redacted]% for NC	
			Interassay %CV (response)	[redacted]% for HPC, LPC, NEC, and NC	
Target titer	**Median titer**	[redacted]			HALO 21075 rev0, Table 6
Titer precision	All titer values are within [redacted] dilution step of the median titer.				HALO 21075 rev0, Table 6
System suitability ranges	NC (OD) ≤ [redacted] [redacted] ≤ NEC (OD) ≤ [redacted] ≤ LPC (OD) ≤ [redacted] ≤ HPC (OD) ≤ [redacted]				HALO 21075 rev0, Table 3
Selectivity	No matrix effect in Chinese and non-Chinese normal matrix.				HALO 21075 rev0, Table 7 and Table 10
Hemolysis effect	No matrix effect in [redacted]% hemolyzed matrix.				HALO 21075 rev0, Table 8

Bioanalytical method validation report name	**Validation for the Detection of Neutralizing Anti-rHuPH20 Antibody in Human [redacted] Plasma Using Hyaluronidase Activity Assay (Module** 5.3.1.4, HALO 21075 rev0)	
Lipemic effect	No matrix effect in lipemic matrix ([redacted] mg/dL of [redacted]).	HALO 21075 rev0, Table 9
Interference	No interference with free hyaluronan was observed up to [redacted] ng/mL.	HALO 21075 rev0, Table 11
Bench-top/process stability	Up to [redacted] hours [redacted] minutes.	HALO 21075 rev0, Table 13
Refrigerator stability	Up to [redacted] hours [redacted] minutes.	HALO 21075 rev0, Table 14
Freeze-thaw stability	Up to [redacted] freeze-thaw cycles at [redacted] °C to [redacted] °C and [redacted] °C to [redacted] °C.	HALO 21075 rev0, Table 12
Long-term storage	Up to [redacted] days at [redacted] °C to [redacted] °C and [redacted] °C to [redacted] °C.	HALO 21075 rev0, Table 15
Method performance in study ARGX-113-1802 (Module 5.3.1.4, 8486-448)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	8486-448, Section 14.1

ELISA=enzyme-linked immunosorbent assay; HA=hyaluronate; HPC=high positive control; HRP=horse radish peroxidase; IgG=immunoglobulin gamma; LPC=low positive control; MRD=minimum required dilution; NA=not applicable; NAb=neutralizing antibody(ies); NC=negative control; NEC=no-enzyme control; NF=normalization factor; no.=number; rev=revision; rHuPH20=recombinant human hyaluronidase PH20; PSCP=plate-specific screening cut point

表 2.7.1-24　rHuPH20 に対する中和抗体検出のための分析法パフォーマンスの要約（スクリーニングアッセイ）－Method #1 及び Method #2 のクロスバリデーション

Bioanalytical method validation report name	**Cross-Validation for the Detection of Neutralizing Anti-rHuPH20 Antibody in Human Plasma ( ) between and (Module** 5.3.1.4, HALO 23115 rev0**)**	
Method cross-validation	Cross-validation of the same ELISA method (enzyme activity assay) at and using spiked matrix and blank matrix samples.	
Parameters	**Cross-validation performance**	**Source location**
Cross-validation results	(%) samples spiked with PC ranging from to µg/mL (including LPC and HPC) or blank samples passed the acceptance criteria for cross-validation samples because they were in % agreement between the and sites.	HALO 23115 rev0, Table 2

ELISA=enzyme-linked immunosorbent assay; HPC=high positive control; LPC=low positive control; rHuPH20=recombinant human hyaluronidase; SH= ; US=United States

表 2.7.1-25　rHuPH20 に対する中和抗体検出のための分析法パフォーマンスの要約－確認段階のバリデーション Method #1

Bioanalytical method validation report name	**Partial Validation/Addition of a Confirmatory Tier in the Validated Method for the Detection of Neutralizing Antibodies to rHuPH20 in Human [redacted] Plasma** **(Module** 5.3.1.4, HALO 24008 rev0) **Selectivity Assessment in CIDP, MG, PV, and ITP Disease State Human Plasma in the Validated Confirmatory Assay for the Detection of Neutralizing Anti-rHuPH20 Antibodies (8377-995 and 8504-470)** **(Module** 5.3.1.4, HALO 24009 rev0)		
Method description	The NAb assay against rHuPH20 is an assay to detect NAb that interfere with rHuPH20 activity to digest HA (coated on ELISA plate). [redacted] is added to detect remaining undegraded HA on the surface. Streptavidin-HRP is used as detection tool. The confirmation assay is an immuno-depletion step based on the use of immunoglobulin-specific recombinant proteins conjugated to magnetic beads to deplete anti-rHuPH20 NAb.		
Material used QCs	PC antibody: Rabbit anti-rHuPH20 IgG fraction, Batch/Lot No.: [redacted]		
Source and lot of reagents	rHuPH20 reference standard, Batch/Lot No.: [redacted]		
MRD	1/[redacted]		
Validation parameters	**Method validation summary**		**Source location**
Determination of the CCP	CCP of [redacted]% signal reduction was determined with [redacted] plasma samples from healthy individuals assuring a [redacted]% FPR.		HALO 24008 rev0, Section 10.1
QC concentrations	HPC: [redacted] µg/mL LPC: [redacted] µg/mL		HALO 24008 rev0, Table 4
Precision of controls	**Confirmatory assay**	**Intraassay % CV (% inhibition)** **Interassay % CV (% inhibition)**	[redacted]% for HPC, LPC [redacted]% for HPC [redacted]% for LPC
Selectivity	Healthy individuals: ([redacted]) [redacted] ([redacted]%) individuals screened and confirmed positive at an LPC level of [redacted] µg/mL and at an HPC level of [redacted] µg/mL.		HALO 24008 rev0, Table 7
	Healthy individuals: ([redacted]) [redacted] ([redacted]%) individuals screened and confirmed positive at an LPC level of [redacted] µg/mL. [redacted] ([redacted]%) individuals screened and [redacted] ([redacted]%) individuals confirmed positive at an HPC level of [redacted] µg/mL.		HALO 24008 rev0, Table 7

Bioanalytical method validation report name	**Partial Validation/Addition of a Confirmatory Tier in the Validated Method for the Detection of Neutralizing Antibodies to rHuPH20 in Human [redacted] Plasma** **(Module** 5.3.1.4, HALO 24008 rev0) **Selectivity Assessment in CIDP, MG, PV, and ITP Disease State Human Plasma in the Validated Confirmatory Assay for the Detection of Neutralizing Anti-rHuPH20 Antibodies (8377-995 and 8504-470)** **(Module** 5.3.1.4, HALO 24009 rev0)	
	Patients with CIDP: [redacted] ([redacted]%) individuals screened and confirmed positive at an LPC level of [redacted] µg/mL. [redacted] ([redacted]%) individuals screened and [redacted] ([redacted]%) individuals confirmed positive at an HPC level of [redacted] µg/mL.	HALO 24009 rev0, Table 4
	Patients with MG: [redacted] ([redacted]%) individuals screened and confirmed positive at an LPC level of [redacted] µg/mL and at an HPC level of [redacted] µg/mL.	HALO 24009 rev0, Table 5
	Patients with PV: [redacted] ([redacted]%) individuals screened and confirmed positive at an LPC level of [redacted] µg/mL. [redacted] ([redacted]%) individuals screened and [redacted] ([redacted]%) individuals confirmed positive at an HPC level of [redacted] µg/mL.	HALO 24009 rev0, Table 6
	Patients with ITP: [redacted] ([redacted]%) individuals screened and confirmed positive at an LPC level of [redacted] µg/mL. [redacted] ([redacted]%) individuals screened and [redacted] ([redacted]%) individuals confirmed positive at an HPC level of [redacted] µg/mL.	HALO 24009 rev0, Table 7
Hemolysis effect	LPC ([redacted] µg/mL) and HPC ([redacted] µg/mL) screened and confirmed positive in [redacted] ([redacted]%) hemolytic samples.	HALO 24008 rev0, Table 9
Lipemic effect	LPC ([redacted] µg/mL) screened and confirmed positive in [redacted] ([redacted]%) lipemic samples. HPC ([redacted] µg/mL) screened positive in [redacted] ([redacted]%) and confirmed positive in [redacted] ([redacted]%) lipemic samples.	HALO 24008 rev0, Table 8
Method performance in ARGX-113-1802 **(Module** 5.3.1.4, 8442-925 addendum 01)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	8442-925 addendum 01, Section 4

CCP=confirmatory cut point; CIDP=chronic inflammatory demyelinating polyneuropathy; CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Fc=crystallizable fragment; FPR=false positive rate; HA=hyaluronidase; HPC=high positive control; HRP=horseradish peroxidase; IgG=immunoglobulin γ; ITP=immune thrombocytopenia; LPC=low positive control; MG=myasthenia gravis; MRD=minimum required dilution; NAb=neutralizing antibody; PC=positive control; PV=pemphigus vulgaris; QC=quality control; rHuPH20=recombinant human hyaluronidase PH20

表 2.7.1-26　rHuPH20 に対する中和抗体検出のための分析法パフォーマンスの要約－確認段階のバリデーション Method #2

Bioanalytical method validation report name	**Partial Validation/Addition of a Confirmatory Tier in the Validation Method for Detection of Neutralizing Anti-rHuPH20 Antibodies in Human [redacted] Plasma using the ELISA** (**Module** 5.3.1.4, HALO 24028 rev0)	
Method description	The NAb assay against rHuPH20 is an assay to detect NAb that interfere with rHuPH20 activity to digest HA (coated on ELISA plate). [redacted] is added to detect remaining undegraded HA on the surface. Streptavidin-HRP is used as detection tool. The confirmation assay is an immuno-depletion step based on the use of immunoglobulin-specific recombinant proteins conjugated to magnetic beads to deplete anti-rHuPH20 NAb.	
Material used QCs	PC antibody: Rabbit anti-rHuPH20 IgG fraction, Batch/Lot No.: [redacted]	
Source and lot of reagents	rHuPH20 reference standard, Batch/Lot No.: [redacted]	
MRD	1/[redacted]	
Validation parameters	**Method validation summary**	**Source location**
Determination of the CCP	CCP of [redacted]% signal reduction was determined with [redacted] plasma samples from healthy individuals, assuring a [redacted]% FPR.	HALO 24028 rev0, Section 5.1.1
QC concentrations	HPC: [redacted] µg/mL LPC: [redacted] µg/mL	HALO 24028 rev0 Table 3
Precision of controls	**Confirmatory assay**: **Intraassay % CV (% inhibition)**: [redacted]% for HPC, LPC **Interassay % CV (% inhibition)**: [redacted]% for HPC, LPC	
Selectivity	No matrix interferences were observed in Chinese normal matrix.	HALO 24028 rev0, Table 4A
Hemolysis effect	No matrix interference was observed in [redacted]% hemolyzed matrix.	HALO 24028 rev0, Table 6
Lipemic effect	No matrix interference was observed in lipemic matrix.	HALO 24028 rev0, Table 8
Method performance in ARGX-113-1802 (**Module** 5.3.1.4, 8486-448 amendment 01)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	8486-448 amendment 01, Section 14.1

CCP=confirmatory cut point; CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Fc=crystallizable fragment; FPR=false positive rate; HPC=high positive control; HRP=horseradish peroxidase; IgG=immunoglobulin γ; LPC=low positive control; MRD=minimum required dilution; NAb=neutralizing antibody; PC=positive control; QC=quality control; rHuPH20=recombinant human hyaluronidase PH20

2.7.1.5　引用文献

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