キネレット皮下注 100 mg シリンジ

＜アナキンラ（遺伝子組換え）＞

第 2 部（モジュール 2）
CTD の概要（サマリー）

2.7　臨床概要

2.7.1　生物薬剤学及び関連する分析法の概要

Swedish Orphan Biovitrum Japan 株式会社

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略語・略号一覧

略語・略号	略していない表現（英語）	略していない表現（日本語）
ADA	Antidrug antibody	抗薬物抗体
AOSD	Adult-onset Still's disease	成人発症スチル病
CAPS	Cryopyrin-associated periodic syndromes	クリオピリン関連周期熱症候群
C_{max}	Maximum concentration	最高濃度
DNA	Deoxyribonucleic acid	デオキシリボ核酸
E. Coli	Escherichia Coli	大腸菌
ECL	Electrochemiluminescence	電気化学発光
EDTA	Ethylenediaminetetraacetic acid	エチレンジアミン四酢酸
ELISA	Enzyme-Linked Immunosorbent Assay	酵素結合免疫吸着測定法
HEK	Human Embryonic Kidney	ヒト胎児腎
HEK-IL-1Ra	Human IL-1 receptor antagonist produced in HEK cells	HEK 細胞で産出されたヒト IL-1 受容体アンタゴニスト
IgG	Immunoglobulin G	免疫グロブリン G
IL-1	Interleukin-1	インターロイキン-1
IL-1R	Interleukin-1 receptor	インターロイキン-1 受容体
IL-1Ra	Interleukin-1 receptor antagonist	インターロイキン-1 受容体アンタゴニスト
IL-1α	Interleukin-1 alpha	インターロイキン-1α
IL-1β	Interleukin-1 beta	インターロイキン-1β
MAT	Mean absorption time	平均吸収時間
MRT	Mean residence time	平均滞留時間
MSD	Meso Scale Discovery	Meso Scale Discovery 社
NAb	Neutralizing antibody	中和抗体
sJIA	Systemic juvenile idiopathic arthritis	全身型若年性特発性関節炎
Sobi	Swedish Orphan Biovitrum AB	Swedish Orphan Biovitrum 社
PK	Pharmacokinetics	薬物動態
t_{max}	Time to maximum concentration	最高濃度到達時間

2.7.1　生物薬剤学試験及び関連する分析法の概要

2.7.1.1　背景及び概観

2.7.1.1.1　緒言

アナキンラ（遺伝子組換え）（ATC コード：L04AC03）は、非糖鎖修飾型の組換えヒトインターロイキン-1 受容体アンタゴニスト（IL-1Ra）であり、N 末端にメチオニン残基が添加されていることを除けばヒト IL-1Ra と同一である。アナキンラはデオキシリボ核酸（DNA）組換え技術を用いて大腸菌（*E.coli*）によって産生されている。薬理学的には、アナキンラが炎症性のサイトカインであるインターロイキン-1（IL-1）（IL-1α 及び IL-1β）のインターロイキン-1 受容体（IL-1R）への結合を競合的に阻害することにより、IL-1 の生物活性を強く阻害し、活動性の炎症を制御する。アナキンラは全身型若年性特発性関節炎（sJIA）及び成人発症スチル病（AOSD）の治療薬として海外で承認を得ており、日本でも同処方の製剤で申請予定である。製剤の詳細は 2.7.1.1.3.2 項に示した。

本概要では、アナキンラは sJIA 及び AOSD を適応症とした日本での開発を目的とした、アナキンラの生物薬剤学試験及び関連する分析法の要約を示す。

2.7.1.1.2　個々の試験結果の要約

アナキンラの開発中に、製剤及び原薬の開発を支持するために、健康被験者を対象に絶対的バイオアベイラビリティ試験を 1 試験（0530 試験）実施した。当該試験は 1991 年に実施した。

アナキンラ製剤の開発中に、[redacted]、[redacted]を製剤に追加した。0530 試験の目的は、旧製剤（[redacted]を含まない）及び新製剤（[redacted]を含む）70 mg を単回皮下投与したときの絶対的バイオアベイラビリティを評価することであった。0530 試験で評価した旧製剤と新製剤の違いは、[redacted]の[redacted]のみであった。新製剤のアナキンラ濃度を 70 から 150 mg/mL に変更した製剤が市販予定製剤である。製剤の詳細は 2.7.1.1.3.2 項に示し、0530 試験の詳細は 2.7.1.2.1 項に示した。

2.7.1.1.3　製剤開発の概要

2.7.1.1.3.1　原薬

19[redacted]～19[redacted]年に実施した臨床試験（0503 試験及び 0530 試験）では第 1 世代製造方法で製造した原薬を使用した。19[redacted]～19[redacted]年に実施した健康被験者及び関節リウマチ患者を対象とした臨床試験では、第 2 世代製造方法で製造した原薬を使用した（表 2.7.1-1）。第 3 世代製造方法の導入後、関節リウマチ、クリオピリン関連周期熱症候群（CAPS）並びに sJIA 及び AOSD 患者を対象としたすべての臨床試験では、現在海外で市販されている製剤と同一の製造方法で製造されたアナキンラの原薬を使用した。現在までに、アナキンラの原薬は 6 世代の製造方法が開発され

ている。表 2.7.1-1 に示した第 1 世代から第 6 世代までの製造方法の変更点は、概して施設移管に伴う軽微な変更のみであった。アナキンラの原薬開発の詳細は［2.3.S.2.6 項］に示した。

表 2.7.1-1　アナキンラの製造方法の概要

Process generation	Manufacturing location	Production years	Main type of supply
Generation 1	[redacted]	19[redacted] to 19[redacted]	Clinical
Generation 2	[redacted]	19[redacted] to 19[redacted]	Clinical
	[redacted]	19[redacted]	Clinical
	[redacted]	19[redacted] to 19[redacted]	Clinical
Generation 3	[redacted]	19[redacted] to 20[redacted]	Clinical and commercial
Generation 4	[redacted]	20[redacted] to 20[redacted]	Commercial
Generation 5	[redacted]	20[redacted] to today	Clinical and commercial
Generation 6	[redacted]	20[redacted] to today	Clinical and commercial

2.7.1.1.3.2　製剤

0530 試験の結果から、アナキンラの絶対的バイオアベイラビリティ及び吸収速度に関して新製剤の皮下投与で改善が認められた。よって、0530 試験完了以降に実施した臨床試験では、アナキンラの賦形剤は、新製剤及び市販予定製剤と同じ組成［140 mmol/L 塩化ナトリウム、10 mM クエン酸、0.1%（w/w）ポリソルベート 80、0.5 mM エチレンジアミン四酢酸（EDTA）ジナトリウム及び注射用水］であった。ただし、含有されたアナキンラの濃度は 10～200 mg/mL（10、30、40、75、150 又は 200 mg/mL）であった。本申請資料中に含まれる試験のうち、0503 試験では旧製剤が使用され、0503 試験以外では新製剤が使用された。

最初のアナキンラのプレフィルドシリンジの市販用製造方法は[redacted]社で立ち上げられ、2001 年に米国で承認された。その後、この市販用製造方法は 20[redacted]～20[redacted] 年に[redacted]に、20[redacted] 年に[redacted]（[redacted]）に移管し、それぞれの施設の設備及び基準に適合させた。その後需要の増加に対応するため、20[redacted]～20[redacted] 年に市販用製造方法を現在の製造施設である[redacted][redacted]に移管し、施設の設備及び基準に適合させた。

施設間の移管時には、製造方法全体及び製造規模を維持した。[redacted]で製造する際には、タンパク質を現実的に可能な限り慎重に取り扱う一般的な戦略を採用し、[redacted]及び[redacted]（[redacted]）への移管を通じて最適化した。

プラセボ対照試験の盲検化のために、プラセボ製剤には実薬製剤と同じ賦形剤を含め、外観も同一とした。

アナキンラの製剤開発に関する詳細は［2.3.P.2 項］に、アナキンラの製造方法の概要は［2.3.P.2.3 項］に示した。

2.7.1.1.4　分析法の概要

アナキンラの臨床開発プログラムの過程で、異なる分析法をバリデーションし、薬物動態（PK）、抗薬物抗体（ADA）及び中和抗体（NAb）の評価に使用した。本項では、Swedish Orphan Biovitrum 社（Sobi）が使用した新しい分析法の概要を中心に示した。

分析法のライフサイクルの詳細は付録の 2.7.1.4 項に示した。

2.7.1.1.4.1　アナキンラ濃度の測定

アナキンラ開発プログラムの初期の臨床試験では、酵素結合免疫吸着測定法（ELISA）を用いてヒト血漿中アナキンラ濃度を測定した。本測定法では、捕捉抗体としてモノクローナル抗アナキンラ抗体を、検出抗体としてビオチン標識したポリクローナル抗アナキンラ抗体を使用し、ストレプトアビジン-西洋ワサビペルオキシダーゼによる呈色を 450 nm で測定した。

301 試験及び 303 試験を含む後期の臨床試験では、ヒト血清中アナキンラの定量には電気化学発光（ECL）アッセイを使用した。このアッセイのバリデーションは［5.3.1.4.8[redacted]/113］に示した。このアッセイでは、ストレプトアビジンでコーティングされた Meso Scale Discovery 社（MSD）プレートを用いた。プレートを 1～2 時間ブロッキングし、洗浄後にビオチン標識した抗 IL-1Ra 抗体溶液をプレートに添加して 1 時間インキュベートした。洗浄後、希釈した血清検体をプレートに添加し、2 時間インキュベートした。洗浄後、スルフォタグ付き抗 IL-1Ra 抗体溶液をプレートに添加し、1 時間インキュベートした。最後の洗浄後、電圧をかけると化学発光シグナルを生成するリードバッファーを添加した。

2.7.1.1.4.2　血清中抗アナキンラ抗体の測定

初期の臨床試験では、IL-1Ra に結合及び/又は IL-1Ra の NAb の評価に複数の解析を用いた。初めに、ELISA を用いて血清検体をスクリーニングした。ELISA では、抗アナキンラ抗体の捕捉のために IL-1Ra を、検出抗体としてビオチン標識したニワトリ由来の抗ヒト免疫グロブリン G（IgG）抗体を使用した。ストレプトアビジン-西洋ワサビペルオキシダーゼの基質を添加したときの呈色反応を分光光度計で測定した。呈色反応は、固定された IL-1Ra に結合した抗体量に比例した。検体の光学密度が閾値（ベースライン検体の平均値に標準偏差の 2 倍を足したもの）を超え、かつベースラインの 2 倍以上に増加した場合、IL-1Ra に結合する抗体が陽性と見なした。スクリーニングアッセイで反応性が確認された検体は、免疫応答をさらに評価することができる確認バイオセンサーアッセイを用いて評価した。一部の検体に対して実施した、アナキンラの NAb の抗体価を決定するためのアッセイでは、生物学的細胞ベースアッセイを用いた。

301 試験及び 303 試験を含む後期の臨床試験では、ECL アッセイを用いて ADA のスクリーニング、確認及び抗体価測定を行う段階的免疫原性戦略を確立した。細胞アッセイは、ECL アッ

セイで陽性が確認された抗体のアナキンラの生物学的効果に対する中和能を検証するために確立した。

ECL アッセイは、ヒト血清検体中の抗アナキンラ抗体及びヒト胎児腎（HEK）細胞で産出された IL-1Ra（HEK-IL-1Ra）に対する抗 IL-1Ra 抗体の測定に使用した。このアッセイのバリデーションは［5.3.1.4.5[redacted]/065］に示した。このアッセイでは、ビオチン標識したアナキンラ、希釈した血清検体及びルテニウムスルフォタグ標識したアナキンラをコーティングしていないプレートに添加し、一晩インキュベート（プレインキュベート）した（確認アッセイでは、非標識のアナキンラを加えて希釈した血清検体も用意した）。プレインキュベートした混合物を、1 時間ブロッキングしたストレプトアビジンでコーティングした MSD プレートに移し、1 時間インキュベートした。最後の洗浄後、電圧をかけると化学発光シグナルを生成するリードバッファーを添加した。

iLite™技術を用いた細胞アッセイを、アナキンラ及び HEK-IL-1Ra に対する NAb の測定に使用した。この方法は 019-002 試験でバリデーションし、019-008 試験で再バリデーションした。使用したアッセイは iLite™ IL-1 Assay Ready であり、用いた細胞は IL-1R 応答性プロモーターによってホタルルシフェラーゼを発現し、構成的プロモーターによってウミシイタケルシフェラーゼを発現するように最適化された細胞であった。IL-1α 又は IL-1β が IL-1R シグナル伝達を活性化させることで、レポーター遺伝子であるホタルルシフェラーゼの発現が亢進される。ホタルルシフェラーゼによるシグナルは、ルシフェラーゼ基質を添加し、インキュベーション後にルミノメーターで測定した。ホタルルシフェラーゼのシグナル強度は、検体中の IL-1α 又は IL-1β によって活性化された IL-1R の機能的活性に比例した。IL-1Ra（アナキンラ又は HEK-IL-1Ra）が存在すると、IL-1β によって誘導されるホタルルシフェラーゼシグナルが減少するが、IL-1Ra に対する NAb が存在すると IL-1Ra に結合することでシグナルが増加する。構成的プロモーターによって発現されるウミシイタケルシフェラーゼは、ホタルルシフェラーゼと同じウェルで、ウミシイタケルシフェラーゼの基質を添加後にインキュベーションして順次測定した。これにより、細胞数のばらつきを補正することで結果を標準化した。

2.7.1.2　全試験を通しての結果の比較と解析

2.7.1.2.1　絶対的バイオアベイラビリティ試験：0530 試験

試験名：インターロイキン-1 受容体アンタゴニストの 2 製剤のバイオアベイラビリティを薬物動態解析により評価した試験

目的：本試験の目的は、2 つのアナキンラ製剤（旧製剤と新製剤）70 mg を単回皮下投与したときの絶対的バイオアベイラビリティを決定することであった。新製剤の旧製剤との相違点は、[redacted]を追加する[redacted]ことのみであった。本試験の治験薬には、第 2 世代の製造方法で製造された原薬が使用されていた。絶対的バイオアベイラビリティの決定には、静脈内投与したときのアナキンラをバイオアベイラビリティの基準（100%）として使用した。本試験で比較したアナキンラの新製剤は、アナ

キンラ濃度を 70 から 150 mg/mL に変更したことを除き、市販予定製剤と同一であった。さらに、[redacted]の濃度が同様であったことから、得られた PK パラメータはアナキンラの PK 特性を裏付けるために使用できると考えられた。

試験デザイン：本試験は、12 例の健康被験者を対象とした非盲検、3 期、クロスオーバーのバイオアベイラビリティ試験であった。

方法：本試験は 1991 年に実施した。被験者は、組入れ時に、[redacted]を含まない旧製剤を 3 時間かけて行う静脈内投与又は皮下投与のいずれかに割り付けられ、各投与群でアナキンラ 70 mg を 1 回投与された。2 回目の投与では、1 回目と異なる投与経路から旧製剤を投与された。投与間には 1 週間のウォッシュアウト期間を設けた。いずれの被験者も 3 回目の投与として新製剤（[redacted]を含む）を皮下投与された。両製剤の組成を表 2.7.1-2 に示す。投与前に、旧製剤（アナキンラ 200 mg/mL）を無菌条件下で 70 mg/mL に希釈した。希釈には、アナキンラを含まない旧製剤と同様の成分のプラセボ製剤を使用した。

表 2.7.1-2　アナキンラの旧製剤及び新製剤の内訳（0530 試験）

Component	Original formulation	New formulation
Anakinra per 1 mL vial	[redacted]mg/mL	70 mg/mL
Sodium chloride	[redacted]mM	140 mM
EDTA	[redacted]mM	0.5 mM
Citric acid	[redacted]mM	10 mM
Polysorbate-80	[redacted]	0.1 % weight/weight

Abbreviation: EDTA, Ethylenediaminetetraacetic acid.

バイタルサイン、血液生化学検査及び抗体価は試験の前後に評価した。投与期間中、PK 評価用のための血液検体を投与後 24 時間にわたり頻回に採取した。各投与期間の間で定期的に以下の評価を実施した。

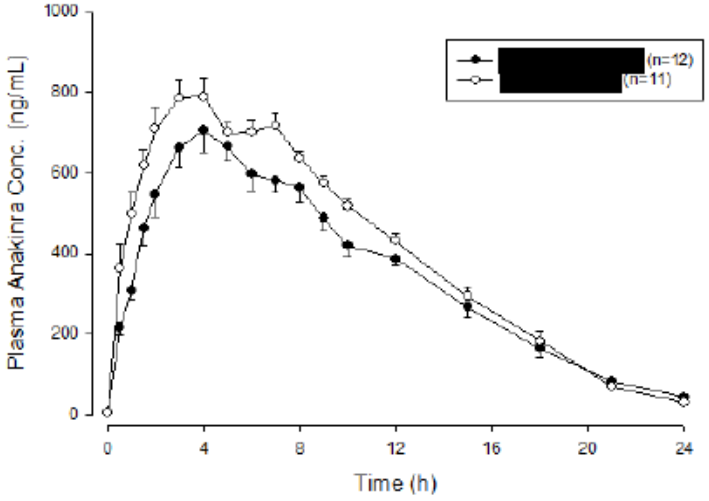
- 有害事象及び併用薬の情報を収集した。
- クレアチニンクリアランスを算出するために、治験薬投与前に血清クレアチニンを測定した。
- 治験薬投与後 0～24 時間に総クレアチニン、クレアチニンクリアランス及びアナキンラ排泄の評価のために尿検体を採取した。

結果：11 例全例がすべての治験薬の投与を完了した。被験者は全例男性で、平均年齢（標準偏差）は 23.8（3.5）歳であった。

本項では、皮下投与後の結果を要約した。静脈内投与した被験者の結果は［2.7.2.2.1.1 項］に要約した。[redacted]を含む新製剤を皮下投与したときの血漿中アナキンラの最高濃度（C_{max}）は、旧製剤と比較して高かったが、最高濃度到達時間（t_{max}）に差は認められなかった（図 2.7.1-1 及び表 2.7.1-3）。絶対的バイオアベイラビリティの平均値（標準偏差）は、旧製剤

［83.2（14.1）%］と比較して[redacted]を含む新製剤［95.4（11.0）%］でやや高かった。吸収速度は新製剤の方が旧製剤よりも高かった。吸収速度の増加は、皮下投与後の平均滞留時間（MRT）が約 8%減少し、平均吸収時間（MAT）が約 10%減少したことによって示された。

図 2.7.1-1　[redacted]の[redacted]別のアナキンラ 70 mg を単回皮下投与後の血漿中アナキンラ濃度-時間プロファイル（0530 試験）



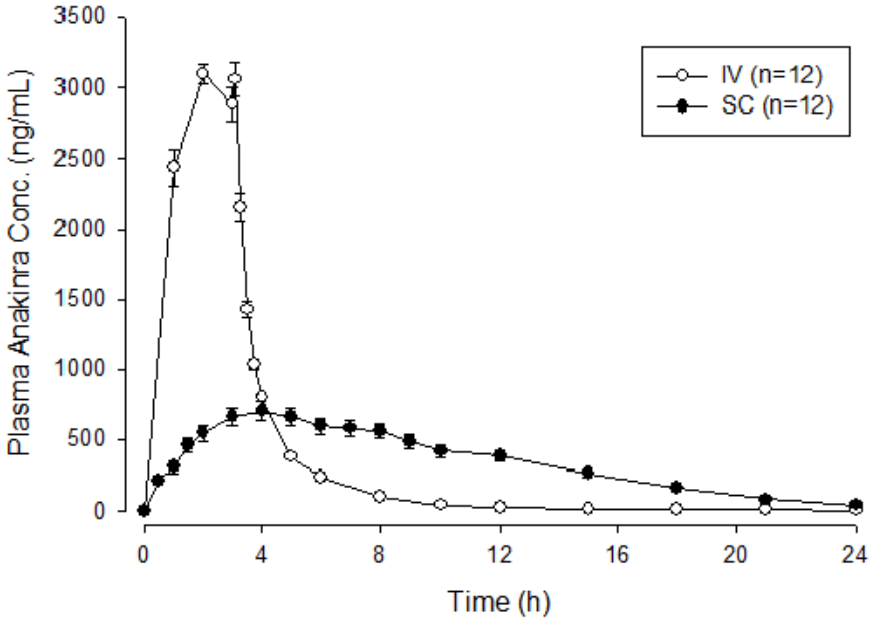
Source: Modified from PK report 0530, Figure 2
Results are mean (standard error of the mean).

表 2.7.1-3　[redacted]の[redacted]別のアナキンラ 70 mg を単回皮下投与後のアナキンラの PK パラメータ（0530 試験）

PK parameter	[redacted]		[redacted]
	i.v. (n=12)	s.c. (n=12)	s.c. (n=11)
C_{max} (ng/mL)	3190 (285)	762 (142)	852 (129)[a]
t_{max} (h)	NA	4.83 (1.70)	4.64 (1.96)
Terminal $T_{1/2}$ (h)	12.3 (5.0)	3.23 (1.07)	2.31 (0.19)[a]
$AUC_{0-\infty}$ (ng x h/mL)	10 400 (1200)	8540 (735)	9730 (667)[a]
CL or CL/F (mL/min)	113 (14)	138 (12)	120 (8)
CL_{cr} (mL/min)	103 (22)	101 (16)	117 (39)
F (%)	100	83.2 (14.1)	95.4 (11.0)[a]
MRT (h)	2.29 (0.64)	9.14 (1.32)	8.39 (0.95)[a]

Source: Modified from PK report 0530, Tables I and II
Abbreviations: $AUC_{0-\infty}$, Area under the concentration-time curve from time zero to infinity; CL, Clearance; CL_{cr}, Creatinine clearance; CL/F, Apparent total clearance of the drug after subcutaneous administration; C_{max}, Maximum concentration; F, Systemic bioavailability; MRT, Mean residence time; NA, Not applicable; PK, Pharmacokinetic; SD, Standard deviation; $t_{1/2}$, Half-life; t_{max}, Time to maximum concentration.
[a]$p<0.05$ (paired Student's t-test).
Results are mean (SD).

図 2.7.1-2 [redacted]を含まないアナキンラ 70 mg を単回静脈内投与又は皮下投与後の血漿中アナキンラ濃度-時間プロファイル（0530 試験）



Source: Modified from PK report 0530, Figure 3
Results are mean (standard error of the mean).

本試験の結果から、健康被験者に 70 mg の用量で単回投与したアナキンラ忍容性は概ね良好であり、各投与群間で安全性の結果に大きな違いはなかった。

結論：PK の結果から、アナキンラの絶対的バイオアベイラビリティ及び吸収速度は、旧製剤と比較して、新製剤の皮下投与で改善が認められた。

2.7.1.3　全試験を通しての結果の比較と解析

実施した生物薬剤学試験は 1 試験のみ（絶対的バイオアベイラビリティ試験 0530 試験）であるため、比較していない。

2.7.1.4 付録

表 2.7.1-4 分析法のライフサイクル

	Method validation [redacted]/113	Clinical study Sobi-ANAKIN-301 Report [redacted]/068	Clinical study Sobi-ANAKIN-303 Report [redacted]/120
Analyte	Anakinra		
Validation type	Full validation	In-study	In-study
eCTD reference number	5.3.1.4.8	5.3.1.4.6	5.3.5.1.2
Method ID	[redacted]/113/v05	[redacted]/113/v04	[redacted]/113/v06
Duration of time method is in use	[redacted]	[redacted]	[redacted]
Bioanalytical site	[redacted]		
Matrix	Human Serum		
Platform	ECL		
Format	A validated sandwich format using anti-IL-1Ra antibodies for capture and detection with electrochemiluminescence readout.		
Stock reference, lot number, expiration date	Anakinra (Kineret) (150 mg/mL, Batch No. [redacted], expiry [redacted] and Batch No. [redacted], expiry [redacted])	Anakinra (Kineret) (150 mg/mL, Batch No. [redacted], expiry [redacted])	Anakinra (Kineret) (150 mg/mL, Batch No. [redacted], expiry [redacted])
Calibration range from the LLOQ to the ULOQ	3.00 ng/mL to 100 ng/mL	3.00 ng/mL to 100 ng/mL	3.00 ng/mL to 100 ng/mL
Matrix study population	Normal	Stills Disease	Japanese patients with newly diagnosed Stills disease
Link to reports and applicable amendments	5.3.1.4.8 Method validation [redacted]/113	5.3.1.4.6 Report [redacted]/068	5.3.5.1.2 Report [redacted]/120
Synopsis of amendment history	NA	NA	NA

Abbreviations: ECL, electrochemiluminescence; eCTD, electronic common technical document; IL-1Ra, interleukin-1 receptor antagonist; LLOQ, lower limit of quantitation; NA, not applicable; UK, United Kingdom; ULOQ, upper limit of quantitation; [redacted]

表 2.7.1-5 分析法のパフォーマンスの概要

Bioanalytical method validation report name, amendments, and hyperlinks	Re-validation of a MSD Method for the Determination of Anakinra in human serum ([redacted]/113)
Method description	A first analytical method (validation study [redacted]/064; report available upon request) was validated and used at the start of clinical trial Sobi-Anakin-401 but was no longer able to meet the batch acceptance criteria due to an increased background following a change to the lot of Blocker Casein buffer used in the method. The format of the assay was changed such that streptavidin coated MSD plates were used instead of standard bind MSD plates (uncoated) and Blocker Casein buffer was no longer used. The immunoassay method for the measurement of anakinra in human serum was re-validated (report [redacted]/113). The assay for determination of anakinra in human serum is an Electrochemiluminescence Immunoassay. Anakinra is a recombinant, non-glycosylated form of the human IL-1Ra, is identical to native human IL-1Ra, except for the addition of an N-terminal methionine residue. The method employs a streptavidin coated MSD plate which is blocked for 1-2 hours. After a wash step, a biotinylated anti-IL-1Ra antibody solution is added to the plate and incubated for 1 hour. After a wash step, diluted serum samples are then added to the plate and incubated for 2 hours. After a wash step, a sulfo-tagged anti-IL-1Ra antibody solution is added to the plate and incubated for 1 hour. After a final wash step, read buffer is added to produce a chemiluminescent signal when an electrical voltage is applied.
Materials used for standard calibration curve and concentration	Anakinra (Kineret) 150 mg/mL, was used for preparation of calibration curves and quality controls Batch No. [redacted], 100% purity, expiry [redacted] 20[redacted] Batch No. [redacted], 100% purity, expiry [redacted] 20[redacted]
Validated assay range	3.00 to 100 ng/mL LLOQ at 2.00 ng/mL was tested during the assay validation but did not meet acceptance criteria.
Material used for QCs and concentration	Anakinra (Kineret) 150 mg/mL, was used for preparation of calibration curves and quality controls Batch No. [redacted], 100% purity, expiry [redacted] 20[redacted] Batch No. [redacted], 100% purity, expiry [redacted] 20[redacted]
MRDs	1/100 dilution
Source and lot of reagents	Biotinylated anti-human IL-1Ra (Cat [redacted]) was obtained from R and D Systems which was stored frozen at -20°C and then stored frozen at -80°C upon reconstitution. Sulfo tag anti-IL-1Ra (CR/[redacted]/00136 or CR/[redacted]/00159) was prepared at [redacted] and was stored frozen at -80°C.
Regression model and weighting	4PL with weighting factor 1/y2

Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	6	Table 1 and 2 of report [redacted]/113
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-3.0 to 6.5%	Table 1 and 2 of report [redacted]/113
	Cumulative precision (%CV) from LLOQ to ULOQ	≤ 10.7%	Table 1 and 2 of report [redacted]/113
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs Intra-batch accuracy for LLOQ at 3.00 ng/mL Intra-batch accuracy from Low QC to ULOQ Inter-batch accuracy from Low QC to ULOQ	-12.3% -9.4 to 4.0% -11.0 to 3.0%	Table 3 to 6 of report [redacted]/113
	Inter-batch %CV	≤ 12.1%	Table 3 to 6 of report [redacted]/113
	Total Error Intra-batch Total Error for LLOQ at 3.00 ng/mL Intra-batch Total Error from Low QC to ULOQ Inter-batch Total Error from Low QC to ULOQ	≤ 24.3% ≤ 28.1% ≤ 23.1%	Table 3 to 6 of report [redacted]/113
Selectivity & matrix effect	As the assay is able to detect endogenous IL-1Ra, it was expected that some unspiked individual sera would not be below the LLOQ. Within acceptance criteria; In total 10 lots were tested, 90.0% of the blank samples were determined below the LLOQ and 80% met bias acceptance criteria when spiked at 2.00 ng/mL.		Table 8 of report [redacted]/113
Selectivity in Japanese matrix	In total 10 lots of serum from healthy Japanese individuals were tested. All blank samples were determined below the LLOQ and 9 out of 10 samples met the bias acceptance criteria when spiked at the LLOQ and all samples met the acceptance criteria when spiked at HQC level.		Table 10 of report [redacted]/120
Interference & specificity	N/A		N/A
Hemolysis effect	Within acceptance criteria; In total 3 aliquots of one hemolysed human control serum were tested, 100% of the blank samples were determined below the LLOQ and 100% met bias acceptance criteria when spiked at 2.00 ng/mL.		Table 9 of report [redacted]/113
Lipemic effect	Within acceptance criteria; In total 3 aliquots of one lipemic human control serum were tested, 100% of the blank samples were determined below the LLOQ and 100% met bias acceptance criteria when spiked at 2.00 ng/mL.		Table 10 of report [redacted]/113
Dilution linearity & hook effect	Dilution linearity within acceptance criteria for dilution factor up to 1/1000; Samples were spiked at 10,000 ng/mL and diluted to 1000, 240, 48.0, 24.0, 12.0, 6.00, 3.00 and 0.300 ng/mL; The observed bias of the nominal ranged from 9.4 to 19.2% for dilution factor up to 1/833.		Table 13 of report [redacted]/113

	Dilution failed to meet acceptance criteria for dilution factor of 1/1666 or 1/3333. Dilution QC (spiked at 10 000 ng/mL and diluted 1/1000) analysed on Intra-batch assessment and in stability experiments met acceptance criteria. There was no hook effect observed, all samples spiked with a concentration >ULOQ had a back-calculated concentration >ULOQ.	Table 3, 15 and 17 of report [redacted]/113 Table 14 of report [redacted]/113
Bench-top/process stability	Demonstrated for 6 hours at room temperature. Storage for 24 hours at room temperature stability failed to meet acceptance criteria.	Table 16 and 17 of report [redacted]/113
Freeze-Thaw stability	Demonstrated for 5 cycles at -80°C.	Table 15 of report [redacted]/113
Long-term storage	Demonstrated for 548 days at -80°C.	Table 18 and 19 of report [redacted]/113
Parallelism	N/A	N/A
Carry over	Not tested, considered not applicable for ligand binding assays	N/A
Method performance in study Sobi.ANAKIN.301 ([redacted]/068)		
Assay passing rate	A first analytical method (validation study [redacted]/064; report available upon request) was no longer able to meet the batch acceptance criteria due to an increased background following a change to the lot of Blocker Casein buffer used in the method. The format of the assay was changed such that streptavidin coated MSD plates were used instead of standard bind MSD plates (uncoated) and Blocker Casein buffer was no longer used. The immunoassay method for the measurement of anakinra in human serum was re-validated (report [redacted]/113). In total, 7 runs were performed and passed acceptance criteria.	Appendix A of report [redacted]/068
Standard curve performance	Cumulative bias range: -7.8 to 5.0% from LLOQ to ULOQ Cumulative precision: ≤ 3.9% CV from LLOQ to ULOQ	Table 2 of report [redacted]/068
QC performance	Cumulative bias range: -13.4 to -9.6% Cumulative precision: ≤ 6.1% CV	Table 4 of report [redacted]/068
Method reproducibility	Incurred sample re-analysis was performed in 21 samples out of 58 (36%), and 81% of the samples met the pre-specified acceptance criteria.	Table 5 of report [redacted]/068
Study sample analysis/ stability	The maximum period of storage of samples was 547 days before analysis falling within demonstrated stability of 548 days.	N/A
Standard calibration curve performance during accuracy and precision runs	N/A	

Method performance in study Sobi.ANAKIN.303 ([redacted]/120)		
Assay Passing rate	4 runs were performed and accepted for partial validation (Selectivity in Japanese serum samples and matrix screening). 7 runs were performed and accepted for sample analysis.	Appendix C of report [redacted]/120
Standard curve performance during sample analysis	Cumulative bias range: -3.1 to 4.7% from LLOQ to ULOQ Cumulative precision: ≤ 6.5% CV from LLOQ to ULOQ	Table 12 of report [redacted]/120
QC performance during sample analysis	Cumulative bias range: -9.1 to -7.6% Cumulative precision: ≤ 6.2% CV	Table 14 of report [redacted]/120
Method reproducibility	Incurred sample re-analysis was performed in 21 samples out of 118 (17.8%), and 71.4% of the samples met the pre-specified acceptance criteria.	Table 16 of report [redacted]/120
Study sample analysis/ stability	The maximum period of storage of samples was 223 days before analysis falling within demonstrated stability of 548 days.	N/A
Standard calibration curve performance during accuracy and precision runs	4 runs were performed and accepted for partial validation (Selectivity in Japanese serum samples and matrix screening). Cumulative bias of calibration curves range: -4.3 to 4.7% from LLOQ to ULOQ	Tables 2, 4 and 7 of report [redacted]/120

Abbreviations: 4PL, 4-parameter logistic; CV, coefficient of variation; HQC, high quality control; IL-1Ra, interleukin-1 receptor antagonist; LLOQ, lower limit of quantitation; MRD, minimum required dilutions; MSD, Meso Scale Discovery; NA, not applicable; QC, quality control; ULOQ, upper limit of quantitation; [redacted]

表 2.7.1-6 ADA スクリーニング、確認及び抗体価測定法の分析法のライフサイクル

Anti-Anakinra antibodies	Method validation [redacted]/065	Clinical Study Sobi-ANAKIN-301 Report [redacted]/069	Clinical Study Sobi-ANAKIN-303 Final Interim Report [redacted]/119
Analyte	Anti-Anakinra antibodies		
Validation type	Full validation	In-study	In-study Interim report
eCTD ref. number	5.3.1.4.5	5.3.1.4.7	5.3.5.1.2
Method ID	[redacted]/065 Version [redacted]	[redacted]/065 Version [redacted]	[redacted]/065 Version [redacted]
Study period	[redacted] to [redacted]	[redacted] to [redacted]	[redacted] to [redacted]
Bioanalytical site	[redacted]		
Matrix	Human Serum		
Platform	Electrochemiluminescence Immunoassay		
Format	A validated bridging format using biotin labelled Anakinra as capture and ruthenium Sulfo-tag labelled Anakinra as detection material		
Stock reference	Anakinra (Kineret) (150 mg/mL, Batch [redacted], expiry [redacted])	Anakinra (Kineret) (150 mg/mL, Batch [redacted] expiry [redacted] and Batch [redacted], expiry [redacted])	Anakinra (Kineret) (150 mg/mL, Batch [redacted], expiry [redacted])
Positive Control	Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted])	Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted])	Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted])
Calibration range	NA		
Matrix study population	Normal	Stills Disease	Japanese patients with newly diagnosed Still's disease
Link to reports	5.3.1.4.5 Method validation [redacted]/065	5.3.1.4.7 Report [redacted]/069	5.3.5.1.2 Final Interim Report [redacted]/119
Synopsis of	NA	NA	NA

amendment history			

Abbreviations: eCTD, electronic common technical document; IL-1Ra, interleukin-1 receptor antagonist; NA, not applicable; UK, United Kingdom; [redacted]

表 2.7.1-7 分析法のパフォーマンスの概要

Method summary		
Bioanalytical method validation report name, amendments, and hyperlinks	Validation of an Analytical Method for the Determination of Anti-anakinra Antibodies in Human Serum (Report [redacted]/065)	
Method description	The presence of anti-anakinra antibodies is determined using a bridging format. Briefly, biotin labelled anakinra, diluted serum sample, and ruthenium Sulfo tag labelled anakinra were incubated overnight in an uncoated plate. The preincubated mixture was then transferred to a streptavidin coated MSD plate, which had been blocked (MSD Blocker A) for 1 hour, and then incubated for 1 hour. After a final wash step, read buffer was added to produce a chemiluminescent signal when an electrical voltage was applied. The instrument measures intensity of the emitted light to afford a semi-quantitative measure of the amount of anti-anakinra antibodies in the sample.	
Materials used for quality controls and concentration	Pool blank matrix (drug-naïve) was used as unspiked Neg-QC; Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted], assumed 100% purity, expiry upon reconstitution [redacted] 20[redacted]), supplied by R and D Systems, was used as the positive control. Anakinra (Kineret) (150 mg/mL, Lot No. [redacted], 100% purity, expiry [redacted] 20[redacted]) was used for spiking in confirmatory assays. Il-1ra (recombinant HEK293 cell produced, Lot No [redacted])	
Minimal required dilutions	4 fold	
Source and lot of critical reagents	Capturing: Biotin labelled Anakinra, produced inhouse from Anakinra lot [redacted] Detection: Rhutenium labelled Anakinra, produced inhouse from Anakinra lot [redacted]	
Regression model and weighting	NA since no calibration curve is included	
Validation parameters	Method validation summary	
Normalization Factor	NF=4.68	Tables 4 and 6; Figures 2 and 3 of report [redacted]/065
Type of Screening Cut-Point	Floating cut-point Plate specific cut point = mean assay negative control + 4.68	

Anakinra Confirmatory Cut-Point	0.8678 A sample with ratio of assay response in the presence of 200 µg/mL anakinra to assay response in the absence of added anakinra less than 0.8678 is confirmed positive for the presence of specific antibodies.	Tables 3 and 5; Figures 4 and 5 of report [redacted]/065
IL-1Ra Confirmatory cut point	0.7250	
Titration Cut-point	NF=8.07 Titration plate specific cut-point= mean assay negative control + 8.07	Tables 4 and 6; Figures 2 and 3 of report [redacted]/065
Sensitivity	Limit of detection of the method was defined as 0.511 ng/mL (low positive control=2.00 ng/mL)	Table 24 Figure 6 of report [redacted]/065
Precision of response (screening assay)	Intra-run precision (%CV) of NC from 3.6 to 9.9% Inter-run precision (%CV) of NC 19.3%	Table 12 of report [redacted]/065
	Intra-run precision (%CV) of LPC (2.00 ng/mL) from 0.6 to 8.5% Inter-run precision (%CV) of LPC 11.3%	
	Intra-run precision (%CV) of MPC (50 ng/mL) from 4.1 to 15.6% Inter-run precision (%CV) of MPC 9.1%	
	Intra-run precision (%CV) of HPC (500 ng/mL) from 3.1 to 15.5% Inter-run precision (%CV) of HPC 9.1%	
Precision of signal reduction for confirmatory assay	Inter-run precision (%CV) at LPC 7.8%	Table 17 of report [redacted]/065
	Inter-run precision (%CV) at MPC 15.0%	
	Inter-run precision (%CV) at HPC 15.1%	
Precision of scoring	All LPC, MPC or HPC were scored positive during screening and confirmatory assays	Tables 12 and 17 of report [redacted]/065
Precision of titers	Precision was acceptable, each individual titer (n=3) did not deviate more than 1 titer step between all runs;	
Prozone (hook effect)	No prozone (hook effect) was observed at concentrations up to 10 000 ng/mL	Table 16 of report [redacted]/065
Drug tolerance (drug interference)	20000 ng/mL for a positive control concentration of 100 ng/mL	Table 17 of report [redacted]/065

Matrix effect in normal serum	The degree of matrix variability of screening assays was acceptable: 8 out of 10 unspiked serum samples scored negative and all the 10 samples spiked at LPC and HPC levels scored positive in screening assay. The confirmation of positive response was within acceptance criteria. 9 out of 10 Neg-QC (unspiked) samples were not confirmed positive by addition of Anakinra and all 10 Low-QC and High-QC samples were confirmed positive by addition of Anakinra.	Table 18 of report [redacted]/065
Established serum stability	Stability of positive control in human serum demonstrated for 24 hours at room temperature	Table 19 of report [redacted]/065
Freeze/thaw stability	Stability of positive control in human serum demonstrated for 6 cycles at -20°C and at -80°C.	Table 20 of report [redacted]/065
Long-term storage	Stability of positive control in human serum demonstrated for 1 months at -20°C and -80°C.	Table 21 of report [redacted]/065
Method performance in study Sobi.ANAKIN.301 ([redacted]/069)		
Bioanalytical report name	Study for the Determination of Anti-anakinra Antibodies in Human Serum Samples from Clinical Study No. Sobi.ANAKIN-301 (the anaSTILLs Study) [redacted]/069	
Source and lot of critical reagents	Pool blank matrix (drug-naïve) was used as unspiked Neg-QC; Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted]) supplied by R and D Systems, was used as the positive control. Anakinra (Kineret) (150 mg/mL, Lot No. [redacted], expiry [redacted]-20[redacted] and Lot [redacted] expiry [redacted]-20[redacted]) was used for spiking in confirmatory assays IL-1Ra (recombinant HEK293 cell produced, Lot No [redacted]) Capturing: Biotin labelled Anakinra, produced inhouse Detection: Rhutenium labelled Anakinra, produced inhouse	
In study Anakinra Cut-point	A new Negative Control was established in [redacted] study [redacted]/067 (Study for the Determination of Anti-anakinra Antibodies in Human Serum Samples from Clinical Study No. Sobi.ANAKIN-401 (the anaGO Study)), and was used also in this study Using this NC the following values and calculations were used. Normalisation factor for screening assay (NF) = 2.305 Plate specific cut point (floating CP) = mean assay NC + 2.305 Titration normalisation factor for screening assay = 5.695 Titration plate specific cut point = mean assay NC + 5.695 A specific study cut-point was not used due to the low number of patients included in the study	Report [redacted]/067

Assay passing rate	17 analytical runs were performed and 13 passed acceptance criteria. While the runs were accepted, 3 runs did not contain any reported results due to incorrect dilution of study samples 2 out of 2 screening runs were accepted. 3 runs for confirmation for anti-anakinra antibodies were preformed and accepted. 2 runs were performed and accepted to check cross-reactivity with IL-1Ra. 16 confirmation runs passed acceptance criteria out of a total of 16 runs; 6 out of 10 titration runs met acceptance criteria.	Appendix A of report [redacted]/069
QC criteria for screening	NC Intra-precision CV ≤14.5%; Inter-precision CV 9.4%; LPC (4 ng/mL) Intra-precision CV ≤14.4%; Inter-precision CV 13.5%; Response ≥SCP HPC Intra-precisionCV ≤11.4%; Inter-precision CV 24.2%; Response ≥SCP	Tables 6 and 7 of report [redacted]/069
QC criteria for confirmation (Anakinra)	LPC CV 4.8% Ratio below CCP HPC CV 12.5% Ratio below CCP	Table 8 of report [redacted]/069
QC criteria for confirmation (IL-1Ra)	LPC Ratio below CCP HPC Ratio below CCP No inter-assay precision was calculated as only one run was performed	Table 9 of report [redacted]/069
Study sample analysis/stability	In total 53 samples were received for analysis. 3 samples could not be analysed due to technical difficulties (samples highly viscous). 21 samples scored positive on screening assay from which 18 were confirmed positive to anti-anakinra antibodies. 12 of the 18 confirmed positive samples were found to cross-react with IL-1Ra. Titers values were obtained for the 18 confirmed positive samples. Sample analysis was performed without established stability data for anti-anakinra antibodies. However testing stability of anti-drug antibodies was considered irrelevant according to EBF recommendation for stability testing of anti-drug antibodies (Pihl et al; Bioanalysis (2014) 6(10), 1409–1413).	Tables 1 to 4 of report [redacted]/069
Changes in method	A confirmatory lot qualification run was performed to validate the use of new batch of Anakinra. This run met acceptance criteria for confirmatory runs	Table 8 of report [redacted]/069

Abbreviations: CCP, confirmatory cut-point; CP, cut point; CV, coefficient of variation; EBF, European Bioanalysis Forum; HEK, human embryonic kidney; HPC, high positive control; IL-1Ra, interleukin-1 receptor antagonist; LPC, low positive control; MPC, medium positive control; MSD, Meso Scale Discovery; NA, not applicable; NC, negative control; NF, normalization factor; SCP, screening cut-point; QC, quality control; [redacted]

表 2.7.1-8 中和抗体の分析法のライフサイクル

Nab antibodies	Method validation 019-002	Method validation 019-008	Clinical Study Sobi-ANAKIN-301 Report 019-003	Method validation 019-014	Clinical Study Sobi-ANAKIN-303 Report 019-012
	表 2.7.1-9	表 2.7.1-10	表 2.7.1-10	表 2.7.1-11	表 2.7.1-11
Analyte	Neutralizing anti-anakinra and anti-IL-1Ra antibodies				
Validation type	Full validation	Full validation	In-study	Partial Validation	In-study
eCTD ref. number	5.3.1.4.1	5.3.1.4.3	5.3.1.4.2	5.3.1.4.4	5.3.5.1.2
Method ID	LS6-S18-v01	TEST-DOC 0282	TEST-DOC 0282	TEST-DOC 0282	TEST-DOC 0282
	N/A	N/A	Version [redacted]	Version [redacted]	Version [redacted]
Study period	[redacted]-20[redacted] to [redacted]-20[redacted]	[redacted]-20[redacted] to [redacted]-20[redacted]	[redacted]20[redacted] to [redacted]-20[redacted]	[redacted]-20[redacted] to [redacted]-20[redacted]	[redacted]20[redacted] to [redacted]-20[redacted]
Bioanalytical site	[redacted]				
Matrix	Human Serum				
Platform	Cell based assay				
Format	A Cell-based assay using Ilite™ IL-1 Ready cells with luminescence detection for testing biological activity of IL-1 and IL-1Ra.				
Stock reference	Anakinra (Kineret) (150 mg/mL, Batch [redacted])	Anakinra (Kineret) (150 mg/mL, Batch [redacted])	Anakinra (Kineret) (150 mg/mL, Batch [redacted])	Anakinra (Kineret) (150 mg/mL, batch [redacted])	Anakinra (Kineret) (150 mg/mL, batch [redacted])
Positive Control	Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted])	Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted])	Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted])	Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted] and [redacted])	Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted] and [redacted])
Matrix study	Normal	Normal	Newly diagnosed Stills	Normal and Japanese	Japanese

population			disease		
Link to reports	5.3.1.4.1 Method validation 019-002	5.3.1.4.3 Method validation 019-008	5.3.1.4.2 Report 019-003	5.3.1.4.4 Method validation 019-014	5.3.5.1.2 Report 019-012
Synopsis of amendment history	NA	NA	N/A	N/A	N/A

Abbreviations: eCTD, electronic common technical document; IL-1, interleukin-1; IL-1Ra, interleukin-1 receptor antagonist; NA, not applicable.

表 2.7.1-9 中和抗体の分析法のパフォーマンスの概要

Method summary		
Bioanalytical method validation report name, amendments, and hyperlinks	Validation report Immunogenicity assay for neutralising antibodies (NAbs) Validation of an assay for the detection of neutralising antibodies (NAbs) towards anakinra and human IL-1 receptor antagonist (IL-1Ra) in human serum Sponsor project number: 019 (Report 019-002)	
Method description	Briefly, the method for neutralizing antibodies is based on the iLite® IL-1 Assay Ready cells which are engineered to express Firefly luciferase under the control of an IL-1 responsive promoter and Renilla luciferase under the control of a constitutive promoter. The Firefly luciferase signal is proportional to the functional activity of IL-1 in the sample. The IL-1 responsive reporter-gene cell line has been optimised for the detection of neutralising anti-anakinra antibodies in human serum. Presence of neutralizing anti-anakinra antibodies will lead to an increased luminescence signal in the samples.	
Materials used for quality controls and concentration	Heat inactivated normal human serum (drug naïve) were used as Background control or as negative control. Pool blank matrix (drug-naïve) was used as unspiked Neg-QC; Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted], assumed 100% purity, expiry upon reconstitution [redacted] 20[redacted]), supplied by R and D Systems, was used as the positive control.	
Minimal required dilutions	N/A	
Source and lot of critical reagents	Anakinra (Kineret) (150 mg/mL, Batch [redacted], expiry [redacted] 20[redacted]) was used for spiking in confirmatory assays. IL-1Ra (Sobi; HEK293 cells produced; 17 mg/ml, batch [redacted]) iLite™ IL-1 Assay Ready Cells (Batch [redacted]) IL-1β (Sobi batch [redacted]); 2.0 mg/mL	
Regression model and weighting	NA since no calibration curve is included	
Validation parameters	Method validation summary	
Screening Cut Point	23% Neutralization	Table 8 of report 019-002
Cross-Reactivity Cut-Point	18% Neutralization	Table 9 of report 019-002
Sensitivity	The sensitivity for the anakinra NAb assay was 332 ng/mL and the sensitivity for the HEK-IL-1Ra NAb assay was 559 ng/mL.	Tables 12 and 13 of report 019-002

Precision Inter-and intraassay	Intra-assay precision anakinra: LQC: 11%CV HQC: 2%CV Inter-assay precision anakinra: LQC: 22%CV HQC: 11%CV Inter-assay precision HEK-IL-1Ra: LQC: 21%CV HQC: 13%CV	Tables 4 and 5, 10 and 11 of report 019-002
Drug tolerance (drug interference)	2500 ng/mL ADA can be measured with 20 ng/mL anakinra present in the sample. 10 µg/mL ADA can be measured with 200 ng/mL anakinra present in the sample.	Tables 18 and 19 of report 019-002
Matrix effect in normal serum	All spiked samples resulted in %N>SCP. No effect on the signal from lipemic samples A low recovery of ADA in haemolytic samples indicates that results of haemolytic samples should be used with care	Tables 20, 21 and 22 of report 019-002
Robustness	No difference in performance between two analysts. No difference between cell density in vials from same cell batch number. Handling of the cells (re-suspension and assay media) can impact ratio BC/NC. Specific recommendations were added to methodological SOP to avoid significant variations between BC/NC ratios.	Tables 17 and 25 of report 019-002
Established serum stability	No reduction in ratios compared to plate controls after storage at room temperature for 24 hours, at 2-8˚C for 168 hours and after 3 freeze-thaw cycles.	Table of report 019-002

Abbreviations: ADA, anti-drug antibody; BC, background, control; CV, coefficient of variation; HEK, human embryonic kidney; HQC, high quality control; IL-1, interleukin-1; IL-1Ra, interleukin-1 receptor antagonist; LQC, low quality control; NA, not applicable; NAb, neutralizing antibody; NC, negative control; SCP, screening cut-point; SOP, standard operational procedure; QC, quality control.

表 2.7.1-10 手法改良の概要

Method summary		
Bioanalytical method validation report name and hyperlinks	Validation of an assay for the detection of neutralising antibodies (NAbs) towards anakinra and human IL-1 receptor antagonist (IL-1Ra) in human serum (Report 019-008)	
Changes in method	The assay was originally validated in study 019-002. Since initial validation, several critical reagents (batch of anakinra, batch of assay ready cells and source of IL-1β standard) had to be changed, making necessary to revalidate the existing method.	
Method description	Briefly, the method for neutralizing antibodies is based on the iLite® IL-1 Assay Ready cells which are engineered to express Firefly luciferase under the control of an IL-1 responsive promoter and Renilla luciferase under the control of a constitutive promoter. The Firefly luciferase signal is proportional to the functional activity of IL-1 in the sample. The IL-1 responsive reporter-gene cell line has been optimised for the detection of neutralising anti-anakinra antibodies in human serum. Presence of neutralizing anti-anakinra antibodies will lead to an increased luminescence signal in the samples.	
Materials used for quality controls and concentration	Heat inactivated normal human serum (drug naïve) were used as Background control or as negative control. Pool blank matrix (drug-naïve) was used as unspiked Neg-QC; Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted], assumed 100% purity, expiry upon reconstitution [redacted] 20[redacted]), supplied by R and D Systems, was used as the positive control.	
Minimal required dilutions	N/A	
Source and lot of critical reagents	Anakinra (Kineret) (150 mg/mL, batch [redacted], expiry [redacted] 20[redacted]) was used for spiking in confirmatory assays. IL-1Ra (HEK293 cells produced; 17 mg/ml,batch [redacted]) iLite™ IL-1 Assay Ready Cells (Ref BM7000 Batch [redacted]) IL-1β, (human rDNA derived; batches [redacted] and [redacted]) WHO international standard 1.0µg	
Validation parameters	Method validation summary	
Screening Cut Point	17% Neutralization	Table 6 of report 019-008
Cross-Reactivity Cut-Point	21% Neutralization	Table 7 of report 019-008
Sensitivity	The sensitivity for the anakinra NAb assay was 410 ng/mL and the sensitivity for the HEK-IL-1Ra NAb assay was 653 ng/mL.	Table 13 of report 019-008

Precision Inter-and intraassay	Intra-assay precision anakinra: LQC: 8%CV HQC: 10%CV Inter-assay precision anakinra: LQC: 23%CV HQC: 12%CV Inter-assay precision HEK-IL-1Ra: LQC: 26%CV HQC: 12%CV	Tables 18 to 21 of report 019-008
Hook effect	All samples with antibody concentration at or above 391 ng/mL gave a positive signal above SCP	Table 13 of report 019-008
Drug tolerance (drug interference)	625 ng/mL ADA can be measured with 20 ng/mL anakinra present in the sample. 2.5 µg/mL ADA can be measured with 200 ng/mL anakinra present in the sample.	Tables 22 and 23 of report 019-008
Matrix effect in normal serum	All spiked samples resulted in %N>SCP. No effect on the signal from hemolytic samples Lipemic samples gave false positive results	Appendix J of report 019-008
Robustness	No difference in performance between two analysts. No difference between cell density in vials from same cell batch number.	Tables 11 and 12 of report 019-008
Method performance in study Sobi.ANAKIN.301 Report 019-003		
Bioanalytical report name	Study Report Analysis of neutralising antibodies (NAbs) towards anakinra and human IL-1 receptor antagonist (IL-1Ra) in human serum in study: SOBI.ANAKIN-301 IND No: [redacted]	
Source and lot of critical reagents	Pool blank matrix (drug-naïve) was used as unspiked Neg-QC; Goat anti-rIL-1Ra polyclonal antibodies (Cat. No. [redacted] 100 µg, Lot No [redacted], assumed 100% purity, expiry upon reconstitution [redacted]-20[redacted]), supplied by R and D Systems, was used as the positive control. Anakinra (Kineret) (150 mg/mL, batch [redacted], expiry [redacted] 20[redacted]) was used for spiking in confirmatory assays. IL-1Ra (HEK293 cells produced; 17 mg/ml, batch [redacted]) iLite™ Il-1 Assay Ready Cells (Ref BM7000 Batch [redacted]) IL-1β, (human rDNA derived; batches [redacted] and [redacted]) WHO international standard 1.0µg	

In-study cut points	SCP 17% Neutralization Cross reactivity cut point 21% Neutralization Cut points determined during validation 019-008 were used in this study. The low number of patients (13), and therefore low number of baseline samples, as well as limitations in planned blood volumes taken at baseline (taking into consideration the expected predominantly low age of patients) did not allow for determining a statistically valid in-study cut point. Also, it was not possible to prepare an “in-patient” serum matrix pool for use as an “in-patient” negative control (”in-patient NC”) in the calculations of % Neutralisation and the NC prepared from normal healthy donor sera was used instead.	
Assay passing rate	1 analytical run was performed and accepted.	
QC criteria for screening	NC Signal Intra-precision CV 5.27% LQC Signal Intra-precision CV 7.30% HQC Signal Intra-precision CV 9.47%	Tables 3 and 4 of report 019-003
Study sample analysis	In total 18 samples, confirmed positive on the ADA assay were analysed on the neutralizing antibody assay. All samples were found Negative for Nabs.	Table 2 of report 019-003

Abbreviations: ADA, anti-drug antibody; CV, coefficient of variation; DNA, deoxyribonucleic acid; HEK, human embryonic kidney; HQC, high quality control; IL-1, interleukin-1; IL-1Ra, interleukin-1 receptor antagonist; LQC, low quality control; NA, not applicable; NAb, neutralizing antibody; NC, negative control; SCP, screening cut-point; QC, quality control; WHO, World Health Organization.

表 2.7.1-11 手法改良の概要

Method summary		
Bioanalytical method validation report name and hyperlinks	Partial Validation of a Bioanalytical Method (TEST-DOC-0282) for determination of Neutralising antibodies (NAbs) against Anakinra and HEK IL-1 Receptor Antagonist (IL-1Ra) in Human Serum (Report 019-014)	
Changes in method	The assay has been validated in studies 019-002 and 019-008. Since the latest validation, several batches critical reagents (batch of anakinra, batch ofHEK-IL1Ra, normal human serum assay ready cells and source of IL-1β standard) had to be changed, making necessary to partially revalidate the existing method.	
Method description	Briefly, the method for neutralizing antibodies is based on the iLite® IL-1 Assay Ready cells which are engineered to express Firefly luciferase under the control of an IL-1 responsive promoter and Renilla luciferase under the control of a constitutive promoter. The Firefly luciferase signal is proportional to the functional activity of IL-1 in the sample. The IL-1 responsive reporter-gene cell line has been optimised for the detection of neutralising anti-anakinra antibodies in human serum. Presence of the antagonist IL-1Ra (Anakinra or HEK-IL-1Ra) will decrease IL-1β induced luciferase signals whereas neutralising antibodies (NAbs) to IL-1Ra will counteract such decrease and increase the signal.	
Materials used for quality controls and concentration	Blank NHS was used as NC. QCs and the BC were prepared by spiking the blank NHS pool with the positive control antibody. Goat anti-Human IL-1Ra/IL-1F3 Antibody (Cat. No. [redacted] 0.2 mg/mL, Lot No [redacted] and [redacted], expiry upon reconstitution respectively 20[redacted] and 20[redacted]), supplied by R and D Systems, was used as the positive control.	
Minimal required dilutions	N/A	
Source and lot of critical reagents	Anakinra (Kineret) (150 mg/mL, batch [redacted], expiry [redacted]-20[redacted]) was used for spiking in confirmatory assays. IL-1Ra (HEK293 cells produced; 0.67 mg/ml, batch [redacted]) iLite™ IL-1 Assay Ready Cells (Ref BM7000 Batch [redacted]) IL-1β, (human rDNA derived; batch [redacted]) WHO international standard 1.0µg	
Validation parameters	Method validation summary	
Screening Cut Point	16% Neutralization	Table 2 of report 019-014
Cross-Reactivity Cut-Point	9% Neutralization	Table 3 of report 019-014
Sensitivity	The sensitivity for the anakinra NAb assay was 651 ng/mL and the sensitivity for the HEK-IL-1Ra NAb assay was 644 ng/mL.	Tables 5 and 6 of report 019-014

Precision Inter-and intra assay	Intra-assay precision anakinra: LQC (750 ng/mL): 11%CV HQC: 7%CV Intra-assay precision HEK-IL-1Ra: LQC (1000 ng/mL): 7%CV HQC: 9%CV Inter-assay precision anakinra: LQC (750 ng/mL): 13% CV ; LQC (800 ng/mL): 14% CV ; LQC (1000 ng/mL): 12%CV HQC: 7%CV Inter-assay precision HEK-IL-1Ra: LQC (750 ng/mL): 18%CV; LQC (800 ng/mL): 32% CV ; LQC (1000 ng/mL): 4%CV HQC: 9%CV	Tables 11, 12, 13 and 14 of report 019-014
Hook effect	All samples with antibody concentration at or above 625 ng/mL gave a positive signal above SCP	Table 7 of report 019-014
Drug tolerance (drug interference)	2500 ng/mL ADA can be measured with 20 ng/mL anakinra present in the sample. 625 ng/mL ADA can be measured with 20 ng/mL HEK-IL-1Ra present in the sample.	Tables 15 and 16 of report 019-014
Matrix effect in human serum	All spiked samples resulted in %N>SCP. No effect on the signal from hemolytic samples Lipemic matrix screened positive for all unspiked and spiked samples. Clinical lipemic samples should be reported with appropriate comment	Tables 8 and 9 of report 019-014
Matrix effect in Japanese matrix	75% of samples spiked to LQC 750 ng/mL screened positive and 100% of unspiked samples screened negative.	Table 10 of report 019-014
Robustness	No difference in performance between two analysts.	Tables 17 and 18 of report 019-014
Method performance in study Sobi.ANAKIN.303 Report 019-012		
Bioanalytical report name and hyperlinks	Interim Study Report Determination of neutralising antibodies (NAbs) towards anakinra (Kineret) and towards human IL-1 receptor antagonist produced in HEK cells (HEK-IL-1Ra) in human serum in the Clinical Study Sobi-ANAKIN-303 (report 019-012)	
Change in assay	N/A	
Source and lot of critical reagents	Blank normal human pooled serum (NHS) was used as NC. QCs and the BC were prepared by spiking the blank NHS pool with the positive control antibody. Goat anti-Human IL-1Ra/IL-1F3 Antibody (Cat. No. [redacted] 0.2 mg/mL, Lot No [redacted], expiry upon reconstitution 20[redacted]), supplied by R and D Systems, was used as the positive control. Anakinra (Kineret) (150 mg/mL, batch [redacted], expiry [redacted]-20[redacted]) was used for spiking in confirmatory assays.	

	IL-1Ra (HEK293 cells produced; 0.67 mg/ml, batch [redacted]) iLite™ IL-1 Assay Ready Cells (Ref BM7000 Batch [redacted]) IL-1β, (human rDNA derived; batch [redacted]) WHO international standard 1.0µg	
Assay passing rate	For sample analysis, 3 analytical runs were performed and accepted for neutralizing anti anakinra and HEK-IL-1Ra antibodies. 1 additional run was performed prior sample analysis and was part of the in-study cut-point determination.	Tables 2, 3 and 4 of report 019-012
In study Cut-point evaluations	Preliminary in-study cut-point evaluations for the Screening and Cross-Reactivity assay were performed in the re-validation study 019-014 where pre-dose samples from all 15 subjects in the present study were analysed in the Screening and Cross-reactivity assay (1 run). To obtain more data, the pre-dose samples were analysed again in this study. All pre-dose samples were negative in the screening and cross-reactivity assay using the cut-points established in 019-014. The cut-points calculated based on the two runs were 0 %N and 1 %N for screening and cross-reactivity, respectively. The calculated in-study cut-points were considered too low to be appropriate for use in the present study. The study samples will be analysed using the cut-points established in 019-014 as it was not possible to determine appropriate cut-points based on the pre-dose sample and as there was not enough material to create a new serum-pool based on the pre-dose samples. SCP 16% Neutralization Cross reactivity cut point 9% Neutralization	Section 10 and Tables 3 and 4 of report 019-012
QC performance for anti-Anakinra Nab	NC Signal Inter-precision CV 22% BC Signal Inter-precision CV 12% LQC %N Inter-precision CV 13% HQC %N Inter-precision CV 11%	Table 6 of report 019-012
QC performance for cross reactivity Nab	NC Signal Inter-precision CV 3% BC Signal Inter -precision CV 8% LQC %N Inter-precision CV 12% HQC %N Inter-precision CV 9%	Table 7 of report 019-012
Study sample analysis	In total 143 samples were received (74 primary and 69 backup) and 39 sample ADA positives were analysed and reported. No samples were reanalysed. All samples were analyzed within the validated freeze-thaw/room-temperature/refrigerated stability. 8 of 39 samples were reported as positive in the screening Anakinra assay. 0 of 8 samples were reported as positive in the cross-reactivity HEK-IL-1Ra assay.	Table 1 and 5 of report 019-012

Abbreviations: ADA, anti-drug antibody; BC, background control; CV, coefficient of variation; DNA, deoxyribonucleic acid; HEK, human embryonic kidney; HQC, high quality control; IL-1, interleukin-1; IL-1Ra, interleukin-1 receptor antagonist; LQC, low quality control; NA, not applicable; NAb, neutralizing antibody; NC, negative control; NHS, normal human pooled serum; SCP, screening cut-point; QC, quality control; WHO, World Health Organization.