No. 26-1333

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

IN RE: ABLYNX N.V., SANOFI,

Appellants,

Appeal from the U.S. Patent and Trademark Office,
Patent Trial and Appeal Board, No. 2026-000193

APPELLANTS' OPENING BRIEF

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April 24, 2026

Claim 51 of U.S. Patent Application No. 17/409,019

51. A fusion protein comprising at least two immunoglobulin single variable domains (ISV), wherein one of the at least two ISVs is at the C-terminal end of the fusion protein, wherein the ISV at the C-terminal end of the fusion protein is a VHH, a humanized VHH, a VH, or a camelized VH that: does not bind to serum albumin; and has a C-terminal end of the sequence $VTVSS(X)_n$ (SEQ ID NO: 34), in which n is 1, 2, 3, 4, or 5, and in which each X is chosen from the group consisting of alanine (A), glycine (G), valine (V), leucine (L), and isoleucine (I), except with the proviso that when n is 3, each X is chosen from the group consisting of glycine (G), valine (V), leucine (L), and isoleucine (I), wherein the fusion protein does not comprise an ISV that binds IL-23 or other interleukins.

CERTIFICATE OF INTEREST

Counsel for Ablynx N.V. and Sanofi certify under Federal Circuit Rule 47.4 that the following information is accurate and complete to the best of their knowledge:

1. **Represented Entities.** Provide the full names of all entities represented by undersigned counsel in this case.

Ablynx N.V., Sanofi

2. **Real Party in Interest.** Provide the full names of all real parties in interest for the entities. Do not list the real parties if they are the same as the entities.

None

3. **Parent Corporations and Stockholders.** Provide the full names of all parent corporations for the entities and all publicly held companies that own 10% or more stock in the entities.

Sanofi, Sanofi Foreign Participations B.V.

4. **Legal Representatives.** List all law firms, partners, and associates that (a) appeared for the entities in the originating court or agency or (b) are expected to appear in this court for the entities. Do not include those who have already entered an appearance in this court.

WOLF, GREENFIELD & SACKS, P.C.: Curtis R. Powell and John R. Van Amsterdam

5. **Related Cases.** Other than the originating case(s) for this case, are there related or prior cases that meet the criteria under Fed. Cir. R. 47.5(a)?

Yes, see separately filed notice

6. **Organizational Victims and Bankruptcy Cases.** Provide any information required under Fed. R. App. P. 26.1(b) (organizational victims in criminal cases) and 26.1(c) (bankruptcy case debtors and trustees). Fed. Cir. R. 47.4(a)(6).

Not applicable

Dated: April 24, 2026

/s/ Daniel J. Minion
Daniel J. Minion

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STATEMENT OF RELATED CASES

No appeal from this proceeding about U.S. Patent Application No. 17/409,019 ("'019 application") assigned to Ablynx N.V. and Sanofi has previously been before this Court or any other court.

Counsel for Ablynx N.V. and Sanofi are aware of several pending cases involving the same legal issue as the instant case, set forth in the following section. Counsel for Ablynx N.V. and Sanofi know of no other cases pending in this or any other court that will directly affect or be affected by this Court's decision here.

PENDING CASES INVOLVING THE SAME LEGAL ISSUE

Ablynx N.V. and Sanofi also hold the rights to U.S. Patent Application Nos. 18/299,871 and 17/704,063, which are each the subject of an appeal to the U.S. Patent and Trademark Office (USPTO) Patent Trial and Appeal Board (Board) involving an obviousness-type double patenting (ODP) rejection: *Ex parte Judith Baumeister et al.*, No. 2026-000190 ("'190 Appeal") and *Ex parte Marie-Ange Buyse et al.*, No. 2026-000685 ("'685 Appeal"), respectively. The '190 and '685 Appeals involve the same legal issue as the instant case. The '190 and '685 Appeals are pending and awaiting a decision by the Board.

Sanofi also holds the rights to U.S. Patent Application No. 17/135,529, which is the subject of an appeal to the Board also involving an ODP rejection. *Ex parte Nicolas Baurin et al.*, No. 2024-002920 ("'920 Appeal"). The '920 Appeal involved

the same ODP legal issue but a different panel of Administrative Patent Judges as the decision appealed here. A Board decision dated November 8, 2024, reversed the ODP rejections of record (*i.e.*, the '920 Appeal Board came to the opposite conclusion on the same ODP legal issue from the panel below), and a request for rehearing made by the Examiner was denied in a decision dated December 18, 2025. On March 5, 2026, the Under Secretary of Commerce for Intellectual Property and Director of the USPTO issued an order convening an Appeals Review Panel and granting rehearing *sua sponte* to review the Board's decisions on appeal and request for rehearing.

JURISDICTIONAL STATEMENT

On November 21, 2025, the Board issued a Decision on Appeal in Case 2026-000193. Ablynx N.V. and Sanofi timely filed a Notice of Appeal of that Decision on January 12, 2026. This Court has jurisdiction over this appeal pursuant to 28 U.S.C. § 1295(a)(4)(A).

INTRODUCTION

The obviousness-type double patenting ("ODP") doctrine is a narrow, judge-made doctrine that is designed to prevent unjust extensions of patent terms. As the patent term framework has evolved, with patent term now defined by statute based on filing date, this Court has repeatedly emphasized and enforced the ODP doctrine's limits, declining to apply ODP where its animating concern is absent. That concern is absent here because no patent term extension is possible.

In 2012, Appellants filed their initial patent application directed to a foundational advance in immunoglobulin single variable domain ("ISV") therapeutics: modifying the exposed C-terminal region of ISVs to reduce interference from pre-existing antibodies and improve overall therapeutic efficacy. The '019 application at issue is a continuation application that claims priority to that initial application, as well as to others in the same patent family. Although filed later, the '019 application is entitled to the earlier application's priority date for patent term purposes. The '019 application is subject to a terminal disclaimer that ensures that the exclusivity on any patent issuing from the '019 application cannot extend beyond its statutory 20-year term.

Appellants (alone and in combination with another entity) subsequently filed separate applications on technologies developed using the foundational technology disclosed in the '019 application. The asserted reference patents claim priority to

these later-filed applications. The claims of these reference patents are undisputedly nonobvious over the ’019 application disclosure and could not have been presented in that application because they relate to distinct inventions that built on the ’019 application’s subject matter. Importantly, each of the reference patents will expire after any patent issuing on the ’019 application.

The Board did not identify any extension of Appellants’ exclusivity periods for the reference patents that would arise from allowing the ’019 application claims because no such extension exists. Nor did the Board allege any gamesmanship. Instead, erroneously believing this Court’s decision in *Fallaux* compelled the outcome, the Board affirmed the ODP rejections of pending claims over reference patents with later patent term filing dates and later expiration dates, relying on a second purported justification for ODP: harassment by multiple assignees. But *Fallaux* does not stand for that proposition. And no other decision of this Court supports applying ODP when no patent term extension is possible, let alone where there is no unjust extension of patent term.

Instead, this Court has repeatedly recognized that the ODP doctrine’s fundamental purpose is to prevent an unjust timewise extension of patent exclusivity and has applied that guiding principle in determining whether a reference qualifies as an ODP reference. Under that principle, the reference patents here do not qualify as ODP references against the ’019 application. Given the ’019 application’s earlier

patent term filing date and the filed terminal disclaimer, a patent issuing on the '019 application cannot extend the term of the patentably distinct reference patents. Accordingly, there is no extension of term here, never mind an unjust one.

STATEMENT OF THE ISSUE

Did the Board err in holding that a later-expiring patent with a later patent term filing date may serve as an ODP reference against a pending application, where the patent and application share no common priority date and the patent claims are undisputedly nonobvious and could not have been presented in the application?

STATEMENT OF THE CASE

I. THE '019 APPLICATION

U.S. Patent Application No. 17/409,019 ("the '019 application") was filed on August 23, 2021. Appx5; Appx159. It is a continuation of U.S. Application No. 14/128,681 ("the '681 application"), filed March 4, 2014, which is a U.S. national stage application under 35 U.S.C. § 371 of international application PCT/EP2012/062251 ("the PCT '251 application"). Appx5; Appx20(p.1:6-15); Appx190. The PCT '251 application was filed June 25, 2012, which is the patent term filing date[1] for the '019 application. Appx5; Appx20(p.1:6-15); Appx190;

[1] For patents that issued from an application filed after the Uruguay Round Agreements Act of 1994, the patent term is 20 years from the filing date of the earliest United States or Patent Cooperation Treaty (PCT) application to which it claims priority, excluding provisional applications ("patent term filing date").

Appx4799, Appx4803. The PCT '251 application published as WO 2012/175741 on December 27, 2012. Appx5. The PCT '251 application claims the benefit under 35 U.S.C. § 119(e) of three U.S. provisional applications filed June 23, 2011 and September 30, 2011, and claims the benefit under 35 U.S.C. § 120 of two PCT applications filed September 30, 2011 and June 14, 2012, and a U.S. application filed March 30, 2012. Appx20(p.1:6-15); Appx190.

Claims 51 and 54-56 of the '019 application are pending and stand rejected only for ODP. Appx2-5; Appx151. The claims relate to a fusion protein comprising at least two immunoglobulin single variable domains (ISVs), wherein one of the at least two ISVs is at the C-terminal end of the fusion protein, and the C-terminal is modified. ISV-based therapies are medicines that can be engineered to target diseases very precisely.

"Conventional" antibodies have antigen-binding sites formed by regions of a heavy chain immunoglobulin variable domain and regions of a light chain immunoglobulin variable domain. In contrast, ISVs are antibody fragments that consist of a single immunoglobulin variable domain; the antigen-binding site being formed by regions of that immunoglobulin variable domain alone. Because ISVs

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Gilead Sci., Inc. v. Natco Pharma Ltd., 753 F.3d 1208, 1211 (Fed. Cir. 2014); Uruguay Round Agreements Act (URAA), Pub. L. No. 103–465, § 532(a), 108 Stat. 4809, 4983–85 (1994).

consist of a single immunoglobulin variable domain, ISVs in isolation (and, *e.g.*, at the C-terminal end of a fusion protein) contain an "exposed" C-terminal region (which would be "buried" in other contexts, such as in the context of a conventional antibody).

The inventors of the instant '019 application found that human subjects may have "pre-existing antibodies" that can bind to ISVs having an "exposed" C-terminal region, thereby potentially decreasing the efficacy of ISV-based therapeutics. Appx24(p.5:30-p.6:14). Pre-existing antibodies are antibodies that can be present in human subjects who have never been administered an ISV. Appx26(p.7:23-30). The inventors also discovered that this interference from pre-existing antibodies can be reduced through certain modifications to an ISV within the exposed C-terminal region. Appx3; Appx55(p.36:1-11). Claim 51, the sole pending independent claim, is directed to a fusion protein comprising at least two ISVs, wherein one of the at least two ISVs is at the C-terminal end of the fusion protein and comprises a C-terminal extension of one to five amino acid residues independently selected from alanine, glycine, valine, leucine, and isoleucine. Appx151. The additional limitations of dependent claims 54–56 are not pertinent to the issue on appeal.

Inventors on the '019 application later developed a further, alternative method for reducing or inhibiting interference by pre-existing antibodies through specific substitution of residues at positions 11 and 89, which can be combined with the

modifications disclosed in the ’019 application. U.S. Patent No. 11,319,364 describes this alternative method and claims ISVs having specific amino acids at these positions. The foundational methods for reducing and/or inhibiting pre-existing antibody binding described in the ’019 application were then subsequently employed in developing several, novel ISV-based therapeutics both internally at Ablynx and also in collaboration with researchers at Merck KGaA. These therapeutics are described and claimed in U.S. Patent Nos. 11,999,797; 11,932,702; 11,603,401; 11,813,307; and 12,129,308. These six follow-on patents, each claiming inventive applications of that foundational technology of the ’019 application, are the references serving as the basis of the ODP rejections at issue here, as discussed below.

II. ODP REJECTIONS AND THE BOARD’S DECISION

The Board affirmed the Examiner’s rejections of all pending claims (claims 51 and 54–56) of the ’019 application as unpatentable for ODP over claims[2] of six patents (“Reference Patents”): U.S. Patent Nos. 11,319,364 (“the ’364 patent”), 11,603,401 (“the ’401 patent”), 11,813,307 (“the ’307 patent”), 11,932,702 (“the

[2] Appellants recognize that *claims* are rejected or held invalid for ODP over reference patent *claims*. For simplicity, this brief refers to the challenged patent applications, challenged patents, and reference patents, instead of specific claims.

’702 patent”), 11,999,797 (“the ’797 patent”), and 12,129,308 (“the ’308 patent”).[3] Appx3-5; Appx18-19; Appx3428-3433. Each Reference Patent is in a different, later-filed patent family than the ’019 application; none of the Reference Patents shares a common priority claim with the ’019 application. Appx5; Appx190; Appx3625; Appx3816; Appx3978; Appx4121; Appx4209; Appx4714; Appx4799.

’364 patent: The ’364 patent was filed on March 29, 2021 and issued on May 3, 2022. Appx3625. It was filed as a continuation of a divisional of a U.S. national stage filing under 35 U.S.C. § 371 of international application PCT/EP2015/060643, filed on May 13, 2015, which is the patent term filing date for the ’364 patent. Appx3625, Appx3739(col.1:3-19); Appx4803. The ’364 patent claims the benefit under 35 U.S.C. § 119(e) of several U.S. provisional applications, the earliest of which was filed May 16, 2014. Appx3625, Appx3739(col.1:3-19).

’401 patent: The ’401 patent was filed on November 26, 2019 and issued on March 14, 2023. Appx3816. It is a U.S. national stage application under 35 U.S.C. § 371 of international application PCT/EP2018/064608, filed on June 4, 2018, which is the patent term filing date for the ’401 patent. Appx3816, Appx3824(col.1:3-10);

[3] Each Reference Patent was cited in a separate rejection of one or more of claims 51 and 54–56 on the ground of ODP; claim 56 was additionally rejected on the ground of ODP as unpatentable over the ’308 patent and over the ’797 patent in view of a second reference, WO 2006/122825 A2 (“Silence”). Appx4. As each rejection and Reference Patent presents the same issue of ODP law, this brief considers the rejections and Reference Patents together.

Appx4803. The ’401 patent claims the benefit under 35 U.S.C. § 119(e) of a U.S. provisional application filed June 2, 2017. Appx3816, Appx3824 (col.1:3-10).

’307 patent: The ’307 patent was filed on November 27, 2019 and issued on November 14, 2023. Appx4121. It is a U.S. national stage application under 35 U.S.C. § 371 of international application PCT/EP2018/064668, filed on June 4, 2018, which is the patent term filing date for the ’307 patent. Appx4121, Appx4131 (col.1:4-11); Appx4803. The ’307 patent claims the benefit under 35 U.S.C. § 119(a) of a European patent application filed June 2, 2017. Appx4121, Appx4131(col.1:4-11).

’702 patent: The ’702 patent was filed on December 17, 2021 and issued on March 19, 2024. Appx3978. December 17, 2021 is the patent term filing date for the ’702 patent. Appx3978; Appx4803. The ’702 patent claims the benefit under 35 U.S.C. § 119(e) of a U.S. provisional application filed December 18, 2020. Appx3978, Appx4004(col.1:6-11).

’797 patent: The ’797 patent was filed on August 5, 2020 and issued on June 4, 2024. Appx4714. It is a U.S. national stage application under 35 U.S.C. § 371 of international application PCT/EP2019/052929, filed on February 6, 2019, which is the patent term filing date for the ’797 patent. Appx4714, Appx4721(col.1:5-14); Appx4804. The ’797 patent claims the benefit under 35 U.S.C. § 119(e) of two U.S.

provisional applications, the earlier of which was filed February 6, 2018. Appx4714, Appx4721(col.1:5-14).

'308 patent: The '308 patent was filed on November 27, 2019 and issued on October 29, 2024. Appx4209. It is a U.S. national stage application under 35 U.S.C. § 371 of international application PCT/EP2018/064667, filed on June 4, 2018, which is the patent term filing date for the '308 patent. Appx4209, Appx4215(col.1:3-10); Appx4804. The '308 patent claims the benefit under 35 U.S.C. § 119(a) of a European patent application filed June 2, 2017. Appx4209, Appx4215(col.1:3-10).

Each Reference Patent shares a common assignee and/or common inventor with the '019 application. Appx5-6; Appx182-188; Appx3625; Appx3816; Appx3978; Appx4121; Appx4209; Appx4714. "[W]hile there are inventors in common between the ['019] application and the reference patents, the inventorship is not identical to any of the reference patents." Appx6 (citing Examiner's Answer at pp.14-15) (cleaned up); Appx4792-4797. The '019 application and Reference Patents are each assigned to one or both of Ablynx N.V. and Sanofi, and the '401, '307, and '308 patents are also assigned to Merck Patent GmbH. Appx1189-1192; Appx3625; Appx3816; Appx3978; Appx4121; Appx4209; Appx4714.

As each Reference Patent was filed after the URAA took effect, the statutory term of each Reference Patent is 20 years from its patent term filing date.[4] *Supra* n.1.

The priority, patent term filing, issue, and 20-year statutory expiration dates for each Reference Patent are summarized below (with corresponding dates for the '019 application included in grey for reference).

	Earliest Priority Date	**Patent Term Filing Date**	**Issue Date**	**20-Year Expiration**
'019 app.	June 23, 2011	June 25, 2012	n/a	June 25, 2032
'364 patent	May 16, 2014	May 13, 2015	May 3, 2022	May 13, 2035
'401 patent	June 2, 2017	June 4, 2018	Mar. 14, 2023	June 4, 2038
'307 patent	June 2, 2017	June 4, 2018	Nov. 14, 2023	June 4, 2038
'308 patent	June 2. 2017	June 4, 2018	Oct. 29, 2024	June 4, 2038
'797 patent	Feb. 6, 2018	Feb. 6. 2019	June 4, 2024	Feb. 6. 2039
'702 patent	Dec. 18, 2020	Dec. 17, 2021	Mar. 19, 2024	Dec. 17, 2041

As each Reference Patent has a patent term filing date that is years later than the '019 application's patent term filing date (Appx5; Appx3625; Appx3816; Appx3978; Appx4121; Appx4209; Appx4714), the 20-year statutory term of any patent issuing on the '019 application would not extend beyond the 20-year statutory term of any Reference Patent. On February 13, 2025, Appellants filed a terminal disclaimer over any part of the statutory term of a patent granted on the '019 application that would extend beyond the expiration dates of U.S. Patent Nos.

[4] Two of the Reference Patents (the '307 and '797 patents) also received a patent term adjustment pursuant to 35 U.S.C. § 154(b) ("PTA"). Appx4121; Appx4714. None of the Reference Patents is subject to a terminal disclaimer that disclaims patent term before the expiration of their respective 20-year statutory terms.

11,192,938 and 10,858,418 (the '938 and '418 patents). Appx3303-3305; Appx4802. That terminal disclaimer was approved on the same day. Appx3306. The '938 and '418 patents issued from applications filed as continuations of the '681 application, the parent of the '019 application, and have the same patent term filing date as the '019 application.[5] Neither the '938 nor '418 patent received PTA.[6] Accordingly, the '938 and '418 patents expire on June 25, 2032, 20 years after the patent term filing date of the '938 and '418 patents and '019 application. The term of a patent issuing on the '019 application therefore would not extend beyond June 25, 2032, even if PTA accrues. *See* 35 U.S.C. § 154(b)(2)(B) ("No patent the term of which has been disclaimed beyond a specified date may be adjusted under this section beyond the expiration date specified in the disclaimer."). By contrast, the earliest any Reference Patent will expire is nearly three years later on May 13, 2035.[7]

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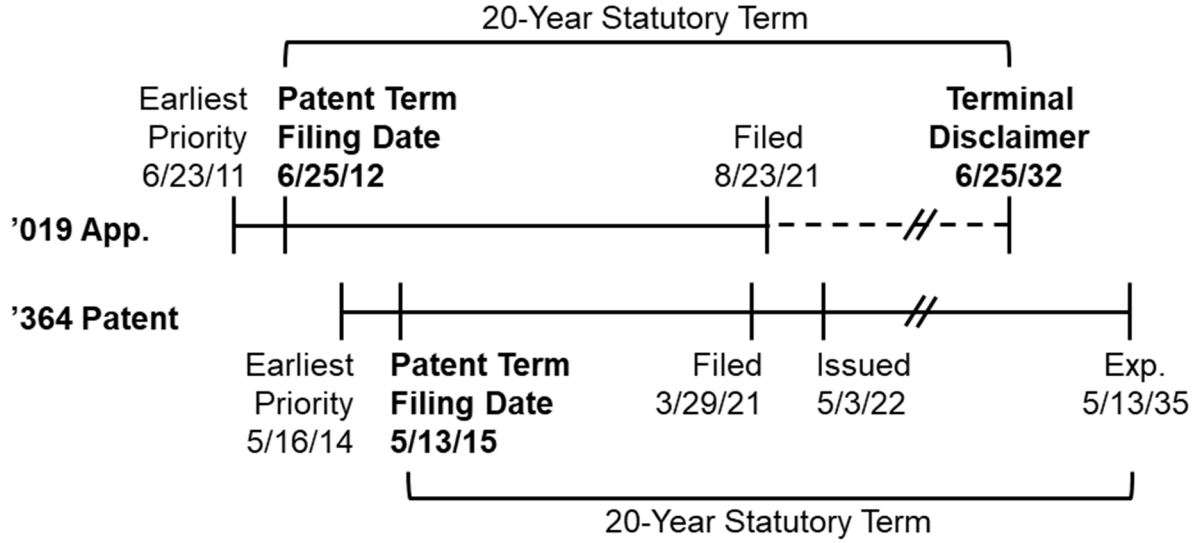
[5] The '938 and '418 patents are not included in the file wrapper of the '019 application; however, under Federal Rule of Evidence 201(b)(2), it would be appropriate for this Court to take judicial notice of these public documents, which are part of the public record of the USPTO. *See, e.g.*, *Standard Havens Prods., Inc. v. Gencor Indus., Inc.*, 897 F.2d 511, 514 n.3 (Fed. Cir. 1990) (taking judicial notice of U.S. Patent Office correspondence which was part of the public record).

[6] *Id.*

[7] As discussed below, even if the term of a patent granted on the '019 application received a patent term extension pursuant to 35 U.S.C. § 156 ("PTE"), that extension would not change the ODP analysis because ODP does not invalidate a validly obtained PTE. *See Novartis AG v. Ezra Ventures LLC*, 909 F.3d 1367, 1373-74 (Fed. Cir. 2018) ("*Ezra*"); *cf. Allergan USA, Inc. v. MSN Labs. Private Ltd.*, 111 F.4th 1358, 1363 n.2 (Fed. Cir. 2024) (the expiration date after the addition of PTE of the patent challenged for ODP was not relevant).

The following figure, which shows the timelines of the ’019 application and ’364 patent, is representative of the relative dates of the ’019 application and Reference Patents in this appeal (each of the other five Reference Patents has a later patent term filing date and expiration date than the ’364 patent):



Appellants do not dispute that the Pending Claims of the ’019 application are not patentably distinct over the claims of the Reference Patents cited by the Examiner. However, the Examiner acknowledges the Reference Patent claims were nonobvious over the ’019 application disclosure and could not have been presented in that application. Appx5; Appx4799. Indeed, the PCT ’251 application, to which the ’019 application claims priority, published on December 27, 2012 (Appx5), more than a year before the earliest priority dates of the Reference Patents. *Supra* pp.7-10. Thus, each of the six Reference Patents claims a distinct invention that utilizes, in part, the foundational technology disclosed in the ’019 application.

SUMMARY OF ARGUMENT

The Board erred in maintaining the Reference Patents qualify as ODP references against the '019 application. On several occasions, in varied factual circumstances, this Court has been presented with the question of whether a reference patent qualifies as an ODP reference. In each case, this Court applied the fundamental purpose of the ODP doctrine—preventing an unjust timewise extension of a patentee's exclusivity—as the touchstone for its decision, finding no ODP when that fundamental purpose was not violated. The Court should do the same here and find that the Reference Patents do not qualify as ODP references against the '019 application.

Allowing claims to issue from the '019 application over the Reference Patents would not violate the fundamental purpose of ODP for two independent reasons: (1) the '019 application is *first*, not *second*, relative to the Reference Patents for ODP purposes, and (2) the '019 application, if granted, will not result in *any* extension of Reference Patent term, much less an unjust one.

The ODP doctrine is meant to prevent patentees from obtaining a *second* patent on a patentably indistinct invention to effectively extend the life of a *first* patent to that subject matter. That cannot happen here. The '019 application claims the benefit of an earlier application having an earlier, first patent term filing date. The Reference Patents, which are each in different patent families from the '019

application and are each directed to distinct follow-on inventions, have later, second patent term filing dates. Applying the reasoning from this Court's precedent, including *Ezra*, *Breckenridge*, and *Allergan* (each discussed in detail below), the '019 application is "first" and the Reference Patents are "second" to that application. The Reference Patents do not qualify as ODP references on that basis alone.

Moreover, there is no extension of patent term here. Any patent issuing on the '019 application will expire before any of the Reference Patents expire. The Board did not find otherwise. Instead, the Board relied on an alleged threat of harassment by multiple assignees as a basis for maintaining the ODP rejection, citing *In re Fallaux*, 564 F.3d 1313 (Fed. Cir. 2009). The Board's reliance on *Fallaux* was misplaced. *Fallaux* did not address—let alone decide—whether a later-filed, later-expiring patent may serve as an ODP reference against an earlier-filed application. The applicant there accepted that the references were proper and argued only for application of the two-way test. Treating *Fallaux* as controlling on an issue it never considered was legal error, particularly here where applying ODP would not prevent any extension of patent term. To the extent the Court finds that *Fallaux* controls the facts here, it should revisit that decision en banc.

STANDARD OF REVIEW

This Court may set aside a Board decision that is "arbitrary, capricious, an abuse of discretion, unsupported by substantial evidence, or otherwise not in

accordance with law." *In re Sullivan*, 362 F.3d 1324, 1326 (Fed. Cir. 2004); 5 U.S.C. § 706(2)(A). ODP is a question of law that this Court reviews *de novo*. *In re Emert*, 124 F.3d 1458, 1460 (Fed. Cir. 1997).

ARGUMENT

I. THE BOARD ERRED BY HOLDING THAT THE REFERENCE PATENTS QUALIFY AS ODP REFERENCES AGAINST THE INSTANT APPLICATION

A. The Fundamental Purpose of the ODP Doctrine Is to Prevent an Unjust Extension of Patent Term

ODP is an equitable doctrine created to prevent patentees from unjustly extending the patent term rights on an invention beyond the original term mandated by Congress. *See Allergan*, 111 F.4th at 1369 ("[T]he purpose of the ODP doctrine . . . is to prevent patentees from obtaining a *second* patent on a patentably indistinct invention to effectively extend the life of a *first* patent to that subject matter.") (emphasis in original).[8] This Court has emphasized that ODP "ensures that a particular invention (and obvious variants thereof) does not receive an undue patent term extension" and "that the public gets the benefit of the invention after the

[8] Unless otherwise noted (such as here), all internal citations and quotations have been omitted, and all emphasis has been added.

original period of monopoly expires." *AbbVie Inc. v. Mathilda & Terence Kennedy Inst. of Rheumatology Tr.*, 764 F.3d 1366, 1373 (Fed. Cir. 2014).[9]

For patents filed before Congress changed the patent term laws under the URAA,[10] this Court and its predecessor, the United States Court of Customs and Patent Appeals (CCPA), applied ODP to prevent patentees from extending the 17-year statutory term mandated by Congress—not to abridge a first patent's 17-year statutory term. "[B]ecause, under the law pre-URAA, the expiration date of the patent was inextricably intertwined with the issuance date, [courts] used the earlier-issued patent to limit the patent term(s) of the later issued patent(s)." *Novartis Pharms. Corp. v. Breckenridge Pharm. Inc.*, 909 F.3d 1355, 1362 (Fed. Cir. 2018) (observing that courts "have applied the principles of obviousness-type double

[9] *See also Gilead*, 753 F.3d at 1210 ("[the] double patenting doctrine prohibits an inventor from extending his right to exclude"); *id.* at 1214 n.5 ("the point of the double patenting doctrine is to protect the public from attempts by inventors to effectively extend their patent term"); *Boehringer Ingelheim Int'l GmbH v. Barr Labs., Inc.*, 592 F.3d 1340, 1346 (Fed. Cir. 2010) ("The policy underlying a double patenting rejection is an important policy because it precludes the *improper extension of the statutory term* of patent protection for an invention."); *Eli Lilly & Co. v. Barr Labs., Inc.*, 251 F.3d 955, 967 (Fed. Cir. 2001) ("Through a statutorily prescribed term, Congress limits the duration of a patentee's right to exclude others from practicing a claimed invention . . . . The judicially-created doctrine of obviousness-type double patenting cements that legislative limitation by prohibiting a party from obtaining an *extension of the right to exclude* through claims in a later patent that are not patentably distinct from claims in a commonly owned earlier patent.").

[10] From 1861 until the June 8, 1995, effective date of the URAA, the term of a United States patent was 17 years from the date the patent issued. Act of Mar. 2, 1861, ch. 88, 12 Stat. 246.; *Gilead Scis., Inc.*, 753 F.3d at 1211.

patenting for over a century to restrict a patent owner's patents on an invention and obvious variants to one 17-year patent term"). Specifically, because the term of pre-URAA patents was based on the issue date, "[a] patentee could file successive continuations and obtain additional patent term for obvious modifications of its earlier claims where its earlier patents and applications did not qualify as prior art, and perhaps do so *ad infinitum*." *Gilead*, 753 F.3d at 1217 (Rader, J., dissenting). "Courts used obviousness-type double patenting to curtail that practice." *Id*.; *Boehringer*, 592 F.3d at 1346 (explaining that for pre-URAA patents, "double patenting is an important check on improper extension of patent rights through the use of divisional and continuation applications"); *Sun Pharm. Indus., Ltd. v. Eli Lilly & Co.*, 611 F.3d 1381, 1387 (Fed. Cir. 2010) (finding that it "would shock one's sense of justice" to allow a patentee to extend its pre-URAA patent rights by securing a second later-expiring pre-URAA patent on obvious subject matter). Where a later-issuing, later-expiring pre-URAA patent sought to extend the 17-year term of a first pre-URAA patent, courts have thus held invalid the later-issuing of the two pre-URAA patents, because a pre-URAA patent's issue date dictated its expiration date.

Following the URAA's enactment,[11] this Court considered whether double patenting could still arise between two post-URAA patents and concluded that it

[11] Under the URAA, the term of a United States patent issuing from an application filed on or after June 8, 1995, was 20 years from the patent term filing date. *Supra* n.1.

could, but that the analysis turns on expiration dates rather than issuance dates. *Gilead* held a later-expiring patent was invalid for ODP where "Gilead crafted a separate 'chain' of applications . . . having a later priority date [and later patent term filing date] than the [reference] patent family," resulting in the issuance of the later-expiring patent-in-suit. 753 F.3d at 1210. *AbbVie* held that ODP could still arise for post-URAA patents where an "applicant chooses to file separate applications for overlapping subject matter and to claim different [patent term filing] dates for the applications, [and thus] the separate patents will have different expiration dates since the patent term is measured from the claimed [effective filing] date." 764 F.3d at 1373. In "such situations," "the doctrine of obviousness-type double patenting ensures that a particular invention (and obvious variants thereof) does not receive an *undue patent term extension*." *Id*. Thus, for post-URAA patents, this Court has continued to apply ODP where it finds an unjust timewise extension of a patentee's exclusivity period.

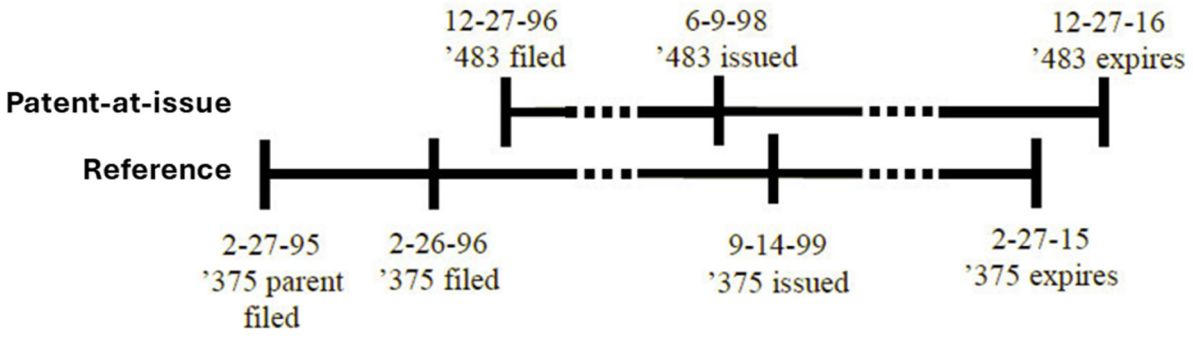
B. This Court Focuses on Whether There Is an Unjust Extension of Patent Term When Deciding the Applicability of ODP

Whether a reference patent qualifies as an ODP reference is a threshold issue in an ODP analysis. *See, e.g.*, *Ezra*, 909 F.3d at 1375 n.4 ("Because we find that the '565 patent is not a double patenting reference for the '229 patent, we need not address Ezra's arguments as to whether the '229 patent is patentably indistinct from the '565 patent."). If a reference does not qualify as an ODP reference, its claims

cannot be used to invalidate the claims at issue for ODP. *Id.*; *Allergan*, 111 F.4th at 1369 ("We therefore hold that a first-filed, first-issued, later-expiring claim cannot be invalidated by a later-filed, later-issued, earlier-expiring reference claim having a common priority date.").

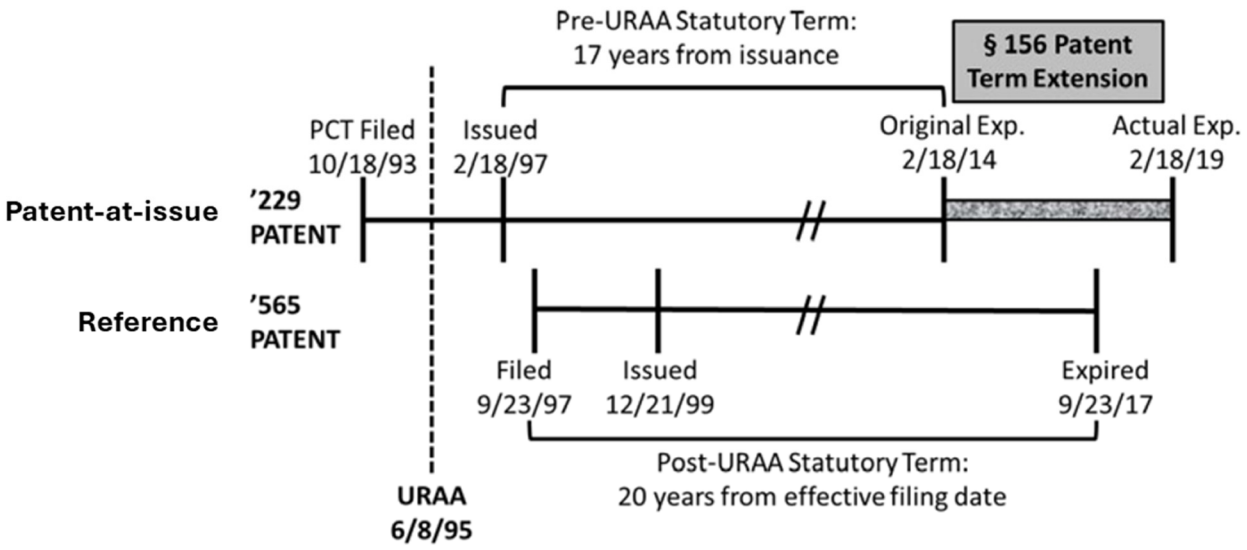
In determining whether a patent qualifies as an ODP reference, this Court has consistently applied the fundamental purpose of the ODP doctrine—preventing an unjust timewise extension of patent protection—as the controlling principle for its determination of whether a reference patent qualifies as an ODP reference.

For example, in *Gilead* this Court considered a "narrow question: Can a patent that issues after but expires before another patent qualify as a double patenting reference for that other patent?" 753 F.3d at 1211-12. And it held that the later-expiring patent was invalid for ODP where "Gilead crafted a separate 'chain' of applications . . . having a later priority date [and later patent term filing date] than the [reference] patent ('375 patent) family," resulting in the issuance of the later-expiring '483 patent. *Id.* at 1210. "Despite their similarities in content," the post-URAA '375 and '483 patents expired at different times because they were not part of the same family of patents and claimed priority to applications having different filing dates. *Id.* The relevant dates for the patents in *Gilead* are shown below.



Id. at 1210 (annotated). This Court reasoned that the '483 patent "extends the inventors' term of exclusivity on obvious variants of the invention claimed in the '375 patent" beyond the expiration of the '375 patent. *Id.* at 1214.

In *Ezra*, this Court considered whether a later-expiring pre-URAA patent having an earlier patent term filing date (the '229 patent) was invalid for ODP because it expired after a post-URAA reference patent having a later patent term filing date (the '565 patent), solely because the '229 patent received patent term extension under 35 U.S.C. § 156. 909 F.3d at 1370, 1373-75. The relevant dates of the '565 and '229 patents are shown below.

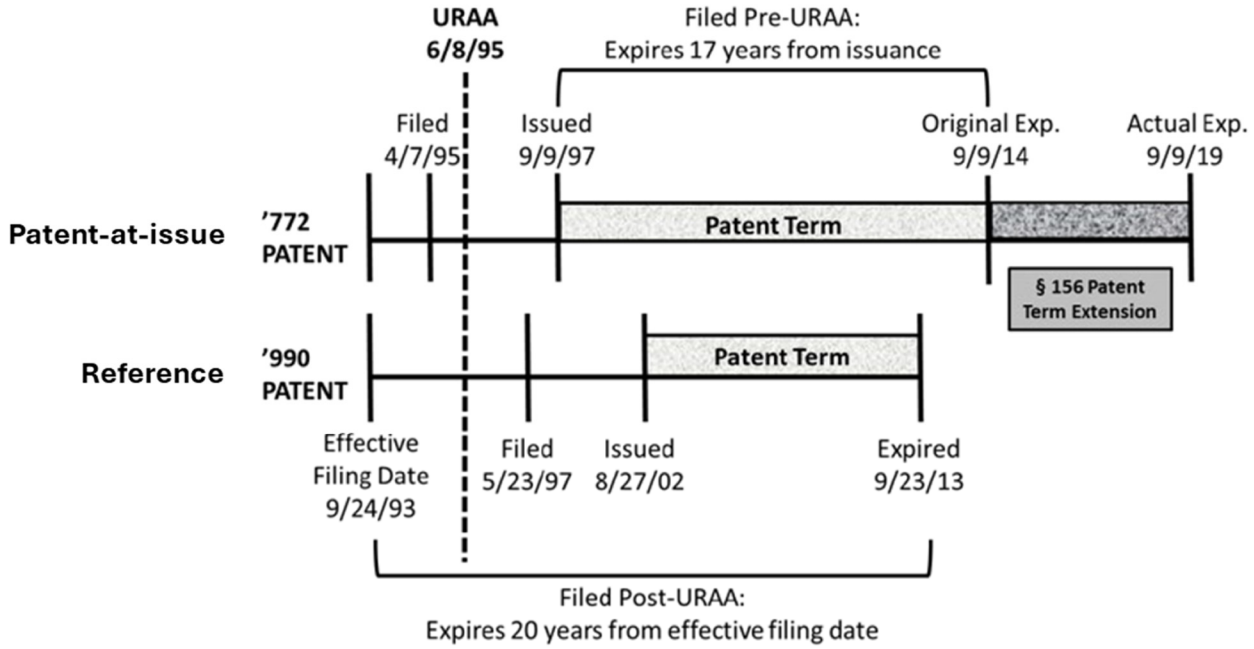


Id. at 1370 (annotated).

Concluding that the later-expiring '229 patent was not invalid for ODP, the Court explained that "[t]his case does not raise the traditional concern with obviousness-type double patenting of a patent owner 'extending his exclusive rights to an invention through claims in a later-filed patent that are not patentably distinct from claims in the earlier filed patent.' *Procter & Gamble Co. v. Teva Pharm. USA, Inc.*, 566 F.3d 989, 999 (Fed. Cir. 2009). Here, it is the earlier-filed, earlier-issued '229 patent, not the later-filed, later-issued '565 patent, that has the later expiration date, due to a statutorily-allowed term extension under § 156." *Ezra*, 909 F.3d at 1374. *Ezra* did not "present the concerns that drove recent decisions of this court regarding obviousness-type double patenting in the post-URAA context" as there was "no potential gamesmanship" to secure an unjust extension of patent term. *Id.* at 1374-75. Accordingly, the Court held "the district court was correct in finding that

the ’565 patent is not a double patenting reference to the ’229 patent and that the ’229 patent is valid through the end of its PTE.” *Id.* at 1375.

In *Breckenridge*, this Court addressed whether a pre-URAA patent (the ’772 patent) was invalid for ODP over a post-URAA patent in the same family (the ’990 patent), having the same patent term filing date, which expired earlier solely due to the change in patent term law. 909 F.3d at 1359. The facts of *Breckenridge* are shown below:

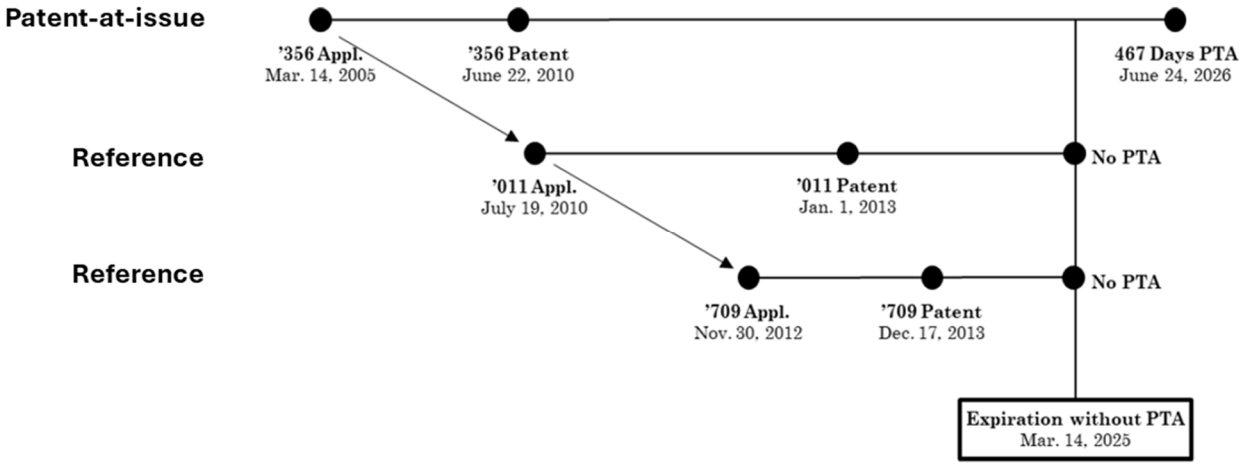


Id. at 1360 (annotated).

Holding that the pre-URAA patent was not invalid for ODP, this Court determined that the post-URAA ’990 patent did not qualify as an ODP reference against the pre-URAA ’772 patent. *Id.* at 1366. The term of the later-expiring pre-URAA patent “d[id] not pose the unjustified time extension problem that was the

case for the invalidated patent in *Gilead*," and the facts of *Breckenridge* did not "give rise to . . . patent prosecution gamesmanship" to obtain an unjust extension of patent term. *Id.* at 1363-64.

More recently, in *Allergan*, this Court faced another ODP question: whether a post-URAA patent having an earlier issue date (the '356 patent) was invalid over two post-URAA patents having the same patent term filing date, later actual filing dates,[12] and later issue dates, which expired first solely because the first-issued patent had received PTA (the '011 and '709 patents). 111 F.4th at 1369. The facts of *Allergan* are illustrated below.



Id. at 1364 (annotated).

This Court concluded that the earlier-filed and earlier-issued but later-expiring '356 patent was not invalid for ODP over the '011 and '709 patents. *Id.* at 1369,

[12] The "actual filing date" of a patent here refers to the filing date of the specific application that issued as that patent, as opposed to the filing date of an application to which it claims priority.

1371. "Applying the fundamental purposes of ODP to these undisputed facts, the claims of the '356 patent do not 'extend or prolong the monopoly [on eluxadoline] beyond the period allowed by law,' *Miller*, 151 U.S. at 198, 14 S. Ct. 310, and therefore are not subject to ODP over the '011 and '709 patents." *Allergan*, 111 F.4th at 1369; *see also id.* (concluding that the asserted reference patents did not qualify as ODP references because "[t]hat is the only conclusion consistent with the purpose of the ODP doctrine"); *id.* at 1369 ("To hold otherwise—that a first-filed, first-issued parent patent having duly received PTA can be invalidated by a later-filed, later-issued child patent with less, if any, PTA—would . . . run afoul of the fundamental purposes of ODP").

In sum, this Court has consistently treated the fundamental purpose of the ODP doctrine—preventing an unjust timewise extension of a patentee's exclusivity—as the controlling principle for determining whether a reference patent qualifies as an ODP reference. As explained in Section I.C, the Court should do the same here and hold that the Reference Patents do not qualify as ODP references against the '019 application.

C. The Reference Patents Do Not Qualify as ODP References

"[T]he purpose of the ODP doctrine," as reiterated in *Allergan*, "is to prevent patentees from obtaining a *second* patent on a patentably indistinct invention *to effectively extend the life of* a *first* patent to that subject matter." *Id.* at 1369.

Applying the Reference Patents as ODP references against the ’019 application would run afoul of this fundamental purpose of ODP for two independent reasons. First, because the ’019 application and Reference Patents are in different families, and the ’019 application has an earlier patent term filing date than each of the Reference Patents, the ’019 application is undeniably “first” relative to those patents for ODP purposes. The term of a patent having an earlier patent term filing date, such as a patent issuing on the ’019 application, cannot *extend* the term of a patent having a later patent term filing date and claiming a patentably distinct invention, such as the Reference Patents, regardless of when the patents expire. Second, a patent granted on the ’019 application will not “effectively extend the life of” the Reference Patents because, due to its earlier patent term filing date and terminal disclaimer, it will expire before any of the Reference Patents expire.

1. The ’019 application is “first”

The PCT ’251 application, from which the ’019 application received its patent term filing date, begins the original patent term on the claimed invention of the ’019 application. Appx5; Appx20(p.1:6-15); Appx190; Appx4799, Appx4803. Each of the Reference Patents is from a different patent family than the ’019 application, has a later patent term filing date, and claims a distinct, later-developed invention rather than the same or obvious variant of the ’019 application’s claimed invention. Appx5; Appx190; Appx3625; Appx3816; Appx3978; Appx4121; Appx4209; Appx4714;

Appx4799. Applying the reasoning from this Court's precedent to the facts here, the '019 application is undoubtedly "first," and the Reference Patents are "second" to that application. *Cf. Allergan*, 111 F.4th at 1369. Stated another way, a patent granted on the '019 application would not be a "second patent" that could extend the patent terms of the Reference Patents.

In *Allergan*, because the challenged '356 patent and '011 and '709 reference patents were members of the same family, having the same patent term filing date, and the reference patents claimed priority to the application that issued as the '356 patent, it made sense to compare actual filing dates and to treat the first (actually) filed and issued patent as the "first" patent and subsequently (actually) filed and issued patents as "second" to that first patent. *Id*. In doing so, the court refused to accept a contrary position that it believed would be "antithetical to the principles of ODP." *Id.*

Here, where the '019 application and Reference Patents are in different families claiming distinct inventions, each with their own patent term filing date, the relevant comparison is between their patent term filing dates. A patent or application having an earlier patent term filing date sets the maximum period of exclusivity for that invention vis-à-vis later-filed patents claiming nonobvious variations, improvements, or implementations of that first invention. Because the '019

application has an earlier patent term filing date, it is undeniably *first* and does not extend any period of exclusivity on the *second* Reference Patents.

In fact, *Ezra* already concluded that a patent having a later patent term filing date did not qualify as an ODP reference against a patent having an earlier patent term filing date, which expired later due to PTE. *Ezra*, 909 F.3d at 1374-75. This case, just like *Ezra*, does not "present the concerns that drove recent decisions of this court regarding obviousness-type double patenting in the post-URAA context" as there was "no potential gamesmanship" to secure an unjust extension of patent term. *Id.* That the challenged patent in *Ezra* had received PTE, and thus expired later, did not change that analysis.

Moreover, the Reference Patents claim distinct, nonobvious inventions developed years later. Treating these later-developed inventions as "first" merely because they issued before the '019 application would run afoul of *Gilead*, which rejected reliance on issue dates as controlling for determining whether a post-URAA reference qualified as an ODP reference against another post-URAA patent. *Gilead* rejected the patentee's argument that the earlier-expiring patent could not be an invalidating reference because it issued later, stating that determining double patenting based on issue dates for post-URAA patents was "simply too arbitrary, uncertain, and prone to gamesmanship." *See Gilead*, 753 F.3d at 1215-16. ODP should not be applied in a way that converts patents issuing from later-filed

applications with patentably distinct claims into "first" patents to bar the issuance of an application with an earlier patent term filing date. Such a result would resurrect the pre-URAA regime where issue dates controlled, which this Court rejected in *Gilead*.

2. The '019 application cannot extend the terms of the Reference Patents

In the instant case, unlike in *Gilead* and *AbbVie*, there is no evidence of gamesmanship resulting in an undue extension of patent term, and the Board made no such finding. The doctrine's core concern with preventing timewise extension is not implicated here. There can be no extension of the patent term at all because a patent issuing on the '019 application would expire earlier than any of the Reference Patents. And, in these circumstances, a terminal disclaimer would have no operative effect on the term of a patent issuing on the '019 application because there is no term to disclaim. Consistent with 35 U.S.C. § 120, applicants are expressly permitted to file continuation or divisional applications claiming priority to an earlier application. Continuing to file such applications on a first invention, while separately pursuing nonobvious variations or improvements building on that invention in patent families with later patent term filing dates, is not gamesmanship but an exercise of the patentee's rights under the statute.

Appellants are not aware of any case involving either pre- or post-URAA patents in which an earlier-expiring patent was invalidated for ODP by a later-

expiring patent.[13] The Board cited two appeals from decisions of the patent office regarding ODP rejections of pending applications, but as explained in Section I.E, those cases involved limited issues not relevant here, and the Court did not consider whether the references qualified as ODP references. In sum, there is no precedent for finding the Reference Patents qualify as ODP references in this circumstance. And doing so would conflict with the fundamental purpose of ODP.

* * *

"Applying the fundamental purposes of ODP" to the facts here, the claims of a patent issuing on the '019 application would "not extend or prolong the monopoly [on the Reference Patent inventions] beyond the period allowed by law . . . and therefore are not subject to ODP over the [Reference Patents]." *Allergan*, 111 F.4th at 1369. "That is the only conclusion consistent with the purpose of the ODP doctrine." *Id.* The Reference Patents therefore do not qualify as ODP references against the '019 application.

[13] The Supreme Court has addressed obviousness-type double patenting on three occasions, and each time has held that a later-expiring patent should be found void for double patenting—not an earlier-expiring patent. S*uffolk Co. v. Hayden*, 70 U.S. 315, 319 (1865); *Miller v. Eagle Mfg. Co.*, 151 U.S. 186, 198 (1894); *McCreary v. Pennsylvania Canal Co.*, 141 U.S. 459, 467 (1891).

D. The Anti-Harassment Theory Does Not Justify Maintaining the Reference Patents as ODP References Against the '019 Application

This Court has recognized that ODP also serves "to prevent multiple infringement suits by different assignees asserting essentially the same patented invention." *In re Hubbell*, 709 F.3d 1140, 1145 (Fed. Cir. 2013). But this anti-harassment justification for ODP has never been the sole basis for this Court finding a patent qualified as an ODP reference—it has only provided secondary support where there was also an extension of patent term. As explained below, the Board erred in relying on the anti-harassment rationale to invalidate claims where the Reference Patents are not proper ODP references.

The anti-harassment rationale underlies the USPTO's rule that terminal disclaimers filed to obviate an ODP rejection of an application must "[i]nclude a provision that any patent granted on that application . . . shall be enforceable only for and during such period that said patent is commonly owned with the application or patent which formed the basis for the judicially created double patenting." 37 C.F.R. § 1.321(c); *In re Van Ornum*, 686 F.2d 937, 944-46 (CCPA 1982) (discussing the history of 37 C.F.R. § 1.321(c)). "[T]he common ownership requirement in a terminal disclaimer serves the purpose to prevent the possibility of multiple suits against an accused infringer by different assignees of patents claiming patentably indistinct variations of the same invention." Appx15-16 *citing Van Ornum*, 686 F.2d at 944–48; MPEP (9th ed. Rev. 01.2024) § 804.02(VI).

The fact that the anti-harassment justification alone is not sufficient to find a patent qualifies as an ODP reference follows from this Court's holdings in *Ezra*, *Breckenridge*, and *Allergan*, which held certain patents did not qualify as ODP reference patents. If the anti-harassment justification alone were sufficient to find a patent qualified as an ODP reference, every patent would qualify against a challenged patent or application because every reference patent is, by definition, a separate patent from the challenged patent (or patent issuing from a patent application) resulting in the theoretical possibility of multiple assignees. But *Ezra*, *Breckenridge*, and *Allergan* show the opposite; the mere existence of multiple patents alone does not result in ODP.

Moreover, the longstanding two-way test,[14] confirms that the alleged risk of harassment from multiple assignees standing alone is not a sufficient basis to establish that a reference qualifies as an ODP reference. Under the two-way test, when certain conditions are met, no ODP rejection is appropriate even though separate ownership remains possible. *See e.g.*, *In re Berg*, 140 F.3d 1428, 1432

[14] Before the passage of the URAA (when expiration was tied to issuance), "when a later filed improvement patent issue[d] before an earlier filed basic invention, a[n ODP] rejection [was] only proper against the claims to the basic invention if the improvement [was] not patentably distinct from the basic invention." *See In re Braat*, 937 F.2d 589, 593 (Fed. Cir. 1991). "The rationale behind this proposition," called the "two-way test," "is that an applicant . . . who files applications for basic and improvement patents should not be penalized by the rate of progress of the applications through the PTO . . . ." *Id.* Where the two-way test applies, no ODP rejection should follow. *Id.* at 595.

(Fed. Cir. 1998); *see also In re Calvert*, 97 F.2d 638 (CCPA 1938). The very existence of this test, which this Court and its predecessor have applied for decades, proves that the possibility of separate ownership alone cannot justify an ODP rejection.

In *Braat*, this Court applied the two-way test and overturned a Board decision affirming an examiner's ODP rejection. 937 F.2d 589, 590 (Fed. Cir. 1991). The Court acknowledged that its holding would effectively result in the extension of patent term; nevertheless, the Court determined that because this extension of term was justified, the double patenting rejection was not appropriate. *Id.* at 594-95 (explaining that "only if the extension of patent right is *unjustified* is a double patenting rejection appropriate"). Because the conditions of the two-way test were met, a terminal disclaimer *was not* required, since once it is determined that an asserted reference does not qualify as an ODP reference, the inquiry ends.

In addition, the anti-harassment justification for ODP makes little sense where, as here, the pending application and reference patents claim *distinct* follow-on inventions and not *indistinct* variations of the *same* invention. The point of the anti-harassment rationale "is to prevent multiple infringement suits by different assignees asserting *essentially the same patented invention*." *Hubbell*, 709 F.3d at 1145; *see also Pope Mfg. Co. v. Gormully & Jeffery Mfg. Co.*, 144 U.S. 248, 250-51 (1892) ("it was obviously not the intention of the legislature to permit several

monopolies to be made *out of one*, and divided among different persons within the same limits"). As the Board itself recognized, "the common ownership requirement in a terminal disclaimer serves the purpose to prevent the possibility of multiple suits against an accused infringer by different assignees of patents claiming *patentably indistinct variations of the same invention*." Appx15-16 *citing Van Ornum*, 686 F.2d at 944–48. But here, the claims of the '019 application and Reference Patents are *not* "essentially the same" or "indistinct versions of" the same invention. The '019 application and Reference Patents stem from *different* patent families without any common priority claim, the claims of the Reference Patents are undisputedly nonobvious over the '019 application claims, and the claims of the '019 application and Reference Patents could not have been presented in the same application. *Supra* pp.7-10, 12. They are different inventions entitled to different patent exclusivities. There is no basis for relying on the anti-harassment justification as the sole ground for finding the Reference Patents qualify as ODP references on these facts.

E. The Board Erred by Finding *Fallaux* Binding; That Opinion Did Not Consider or Decide the Issue Here

In concluding that the Reference Patents qualify as ODP reference patents against the '019 application, the Board principally relied on a single case: *In re Fallaux*, 564 F.3d 1313. Appx6-9; *see also* Appx16-17. The Board erred by treating *Fallaux* as controlling. In *Fallaux*, the applicant did not raise the question of whether the cited reference patents qualify as ODP reference patents against the application,

and this Court did not decide that issue. *Fallaux* does not resolve the question presented by this appeal.

The Board found that because "[l]ike this case, the reference patents in *Fallaux* have later patent term filing dates compared to the application under examination," and the reference patents and application under examination were all filed post-URAA, "any patent issued from the application would expire before the reference patents expired." Appx8-9 *citing Allergan*, 111 F. 4th at 1367. The Board reasoned that "the Federal Circuit upheld the Board's decision, affirming the [ODP] rejection of the pending claims over the reference patents with later patent term filing dates and later expiration dates because 'there is a second justification for obviousness-type double patenting—harassment by multiple assignees.' *Fallaux*, 564 F.3d at 1318." Appx9. Believing it was bound to do so by *Fallaux*, the Board rejected "Appellant's argument that the reference patents do not qualify as [ODP] references. . . ." Appx9.

The Board, however, overlooked that the question of whether the cited patents qualified as ODP reference patents against the application in *Fallaux* was not raised by the applicant or decided by the Court in that case. The applicant in *Fallaux* did not challenge whether the reference claims used to invalidate the application claims qualified as ODP reference claims. The *only* argument made to the Court in *Fallaux*

was whether the applicant could rely on the two-way test[15] to overcome the ODP rejection made against his claims. *Fallaux*, 564 F.3d at 1315 ("Dr. Fallaux attempted to overcome the rejection by arguing that the examiner should have applied the two-way test for obviousness-type double patenting. . . .On appeal to the Board, Dr. Fallaux argued only that he was entitled to the two-way test."); *id.* at 1316 ("Dr. Fallaux timely appealed, arguing that he is entitled to rely on the two-way test."). Under the principle of party presentation, this Court did not consider whether the cited patents qualified as ODP reference patents against the *Fallaux* application. *Cf. Allergan*, 111 F.4th at 1368-69 (distinguishing *Cellect* on the basis that it did "not address, let alone resolve, any variation of the question presented [in *Allergan*] . . . and therefore has little to say on the precise issue before [the Court]"); *see also id.* at 1369 n. 6 (holding the Court had not considered an issue not challenged by the patent owner in another case, *citing Greenlaw v. United States*, 554 U.S. 237, 243 (2008) ("[W]e rely on the parties to frame the issues for decision and assign to courts the role of neutral arbiter of matters the parties present."")). Accordingly, the Board was not bound by *Fallaux* to hold that the Reference Patents qualify as ODP reference patents, and it erred by holding otherwise. As *Fallaux* did not consider that

[15] Under the two-way test, the examiner must show that the reference claims are not patentably distinct over the application claims. *Fallaux*, 564 F.3d at 1315. In *Fallaux*, the examiner agreed that under the two-way test, the application claims would not have been subject to an ODP rejection. *Id.*

issue, the statements in *Fallaux* regarding the anti-harassment rationale have little bearing "on the precise issue" in this appeal. *Cf. Allergan*, 111 F.4th at 1368-69.

In its discussion of the equities, the Board noted that after *Fallaux*, "the [Federal Circuit] applied the multiple-assignee-harassment rationale again" in *Hubbell*, 709 F.3d at 1147–48. Appx17. But *Hubbell* did not address or decide the question presented here either; the sole issues in *Hubbell* were whether common ownership is a requirement for ODP (the inventive entities were not identical and the applications were never commonly owned) and whether certain alternatives to ODP were available under equity. *See Hubbell*, 709 F.3d at 1144-45 (summarizing Hubbell's arguments on appeal). Thus, *Hubbell* did not address, much less decide, whether the reference patents qualified as ODP references in that case.

The Board further relied on *Cellect*'s statements that "the Board [in *Cellect*] did not err in determining that a risk of separate ownership existed and, even in the absence of separate ownership, that a terminal disclaimer would have been required to ensure common ownership" and "[w]hile [the patent owner] has not engaged in actions that resulted in divided ownership in the past, and it has promised that it will not do so in the future, neither fact suffices to abrogate the potential future risk of multiple owners or assignees." Appx17 *citing In re Cellect, LLC*, 81 F.4th 1216, 1230 (Fed. Cir. 2023). But *Cellect* addressed yet another, different ODP question: what expiration date applies to ODP on a patent that has received PTA. *Cellect*, 81

F.4th at 1223-29. As this Court explained in *Allergan*, "the patent owner in *Cellect* did not challenge whether the reference claims used to invalidate the asserted claims were proper ODP reference claims . . . . [T]he court did not consider that issue." *Allergan*, 111 F.4th at 1369 n.6.

F. Policy Considerations Weigh Strongly Against an Extension of the ODP Doctrine to the Facts of this Case

Patent owners are typically in the best position to develop follow-on technologies covered by their patents, including because the patent owners have freedom to operate with respect to the patents that they own. Allowing a patent having a later patent term filing date to qualify as an ODP reference to a patent (or patent application) having an earlier patent term filing date would disincentivize patent owners from developing patentably distinct follow-on technologies alone and in collaboration with others.

Continuing research and collaboration are key to many innovations. But requiring a terminal disclaimer to overcome ODP over a follow-on patent would require foundational patents to be commonly owned with patents on future innovations to be enforceable. Such a rule would limit innovators from collaborating with others on follow-on inventions, lest common ownership be destroyed and rights to the foundational patent extinguished. Indeed, such is the case here, where certain Reference Patents were developed in collaboration with Merck and are not commonly owned with the '019 application, therefore terminal disclaimers over the

relevant Reference Patents cannot be filed. *Supra* p.9. Patenting these later technologies (*e.g.*, to help recoup costs invested to develop the later technologies) would have the potential to damage patent rights covering the earlier-developed foundational technologies, if the Board is affirmed.

ODP also restricts patent owners' ability to alienate their patent rights covering the later-developed patentably distinct technologies (*i.e.*, because of common ownership requirements for terminal disclaimers). This restriction can have significant real-world consequences. For example, a small inventor that develops foundational technology may lack the resources to bring multiple therapeutics to market and may instead fund development of one product by licensing or selling rights to others to pursue distinct follow-on therapies utilizing the foundational technology. Applying ODP to require common ownership across such inventions would impede those transactions, limiting the ability of innovators to finance development and reducing the number of therapies that reach patients.

The public interest in favor of Appellants strongly outweighs any public benefit to applying ODP on the instant facts. As explained above, a patent issuing on the '019 application would not extend, let alone unjustly extend, the exclusivity on the Reference Patent inventions beyond the period allowed by law. *Supra* Section I.C. The Board did not identify any concern about an unjust timewise extension; the only concern raised by the Board was a hypothetical possibility of

harassment by multiple assignees. Appx15-17. However, prior to *Van Ornum*, the CCPA expressed skepticism about the policy concern that allowing indistinct inventions to issue would lead to harassment by multiple assignees.

> [Issuance of multiple patents] is a very common situation existing with respect to genus and species, dominant and subservient, and 'overlapping' patents, whenever there are unobvious differences, all granted in strict accordance with law and presumed valid. Yet we do not see the courts bogged down with harassment suits. In those rare instances where there is a situation which a court can be persuaded amounts to harassment, it has means for dealing with it by inflicting attorney's fees. This can be a powerful deterrent.

In re Jentoft, 392 F.2d 633, 641 (CCPA 1968); *see also In re Eckel*, 393 F.2d 848, 857 (CCPA 1968). In other words, genus and species and other "overlapping" patents not subject to ODP rejections are very common, so the same argument of harassment of an alleged infringer by multiple assignees could apply to such patents. Yet, as far as Appellants are aware, the courts have not voiced a similar public policy concern about such patents. And the concern of harassment by multiple assignees even for patents in the same family on obvious variants of the same invention does not appear to be supported by any real-world evidence.

II. IF THIS COURT'S PRIOR *FALLAUX* DECISION COMPELS AFFIRMANCE, THIS COURT SHOULD TAKE THE OPPORTUNITY TO REVISIT THAT DECISION

If this Court determines that *Fallaux* is binding precedent that addresses the issue here, Appellants respectfully submit that *Fallaux* was wrongly decided.

Fallaux relies on two CCPA cases, *Van Ornum* and *In re Griswold*, for its discussion of the anti-harassment rationale. *See Fallaux*, 564 F.3d at 1319. Those cases identify only a single Supreme Court ODP decision that purportedly supports an anti-harassment rationale, *Underwood v. Gerber*, 149 U.S. 224 (1893). But *Underwood* did not endorse (or even address) an anti-harassment rationale for ODP. At best, *Underwood* summarized a lower court's decision that may have considered an anti-harassment rationale for ODP. *See id.* at 227-28. That does not mean the Supreme Court accepted the lower court's reasoning: the Supreme Court "review[s] judgments of the lower courts, not statements in their opinions." *Amgen Inc. v. Sanofi*, 598 U.S. 594, 615 (2023).

The Court's discussion of multiple assignee harassment in *Van Ornum* did not establish harassment as an independent basis for ODP. To the contrary, the Court made clear that ODP doctrine is grounded in preventing unjustified extensions of patent terms. *See Van Ornum*, 686 F.2d 937, 943-44 ("The fundamental reason for the rule (against 'double patenting') is to prevent unjustified timewise extension of the right to exclude granted by a patent no matter how the extension is brought about."). Applying that principle, the Court found the rejection was warranted because the claims on appeal were encompassed in the inventions claimed in the earlier patents, such that practicing the earlier patents would fall within the scope of the later claims. *Id.* at 944. The Court's subsequent discussion of multiple assignee

harassment related solely to its analysis of whether a terminal disclaimer could overcome an already justified ODP rejection. *See id.* ("[T]he next question is whether appellants can overcome the rejection by filing a terminal disclaimer.").

Griswold's discussion of multiple assignee harassment was limited to *dicta* in a footnote noting the applicant's "imaginative solution [in terminal disclaimers] to one of the more theoretical objections to double patenting, split ownership of two patents and potential harassment." *In re Griswold*, 365 F.2d 834, 840 n.5 (CCPA 1966). The Court gave the terminal disclaimers "no effect," finding the inventions claimed in the challenged application and reference patent were "the same." *Id.*

Underwood's passing and ambiguous digest of a lower court's decision cannot outweigh the otherwise substantial backing for not considering patents or applications with later patent term filing dates and later expiration dates than the challenged claims to be valid ODP references. Neither *Van Ornum* nor *In re Griswold* support the Board's position either. For the reasons explained above, holding a patent having a later patent term filing date qualifies as an ODP reference against a patent having an earlier patent term filing date is at odds with the fundamental purpose of ODP, particularly where the reference patents expire *after* the challenged patent (or a patent issuing on a challenged application) and claim patentably distinct inventions.

Accordingly, to the extent *Fallaux* can be read to address the issue presented here (Appellants do not believe that it can), *Fallaux* should be reconsidered by this Court sitting en banc.

CONCLUSION

For the foregoing reasons, the Board erred in affirming the Examiner's rejections of claims 51 and 54–56 for ODP. As the Board's decision was premised on its determination that the Reference Patents qualified as ODP references against the '019 application, and that determination was wrong for the reasons explained herein, the judgment affirming the Examiner's rejections of the '019 application claims should be reversed.

Dated: April 24, 2026

Respectfully submitted,

/s/ Daniel J. Minion

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ADDENDUM
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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/409,019	08/23/2021	Judith Baumeister	A0848.70142US12	4367

159933 7590 11/21/2025

Sanofi (Bioverativ/Ablynx/Pasteur)/Wolf
c/o Wolf, Greenfield & Sacks, P.C.
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EXAMINER	
ALLEN, MARIANNE P	

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
11/21/2025	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte JUDITH BAUMEISTER, MARIE-PAULE LUCIENNE ARMANDA BOUCHE, CARLO BOUTTON, MARIE-ANGE BUYSE, VEERLE SNOECK, STEPHANIE STAELENS, BRUNO DOMBRECHT, PETER SCHOTTE, CEDRIC JOZEF NÉOTÈRE VERVERKEN, GERALD BESTE, GUY HERMANS, SOREN STEFFENSEN, ALEXANDER SZYROKI, and TINNEKE DENAYER

Appeal 2026-000193
Application 17/409,019
Technology Center 1600

Before JEFFREY N. FREDMAN, ZHENYU YANG, and RUSSELL E. CASS, *Administrative Patent Judges*.

YANG, *Administrative Patent Judge*.

DECISION ON APPEAL

Pursuant to 35 U.S.C. § 134(a), Appellant[1] appeals from the Examiner's decision to reject claims 51 and 54–56. We have jurisdiction under 35 U.S.C. § 6(b).

We AFFIRM.

[1] "Appellant" refers to "applicant" as defined in 37 C.F.R. § 1.42. Appellant identifies the real party in interest as Ablynx N.V. and Sanofi. Appeal Br. 3.

STATEMENT OF THE CASE

The Specification states that "the present invention provides methods and assays that easily allow the skilled person to predict whether an immunoglobulin single variable domain will or will not have a tendency to undergo a[]specific protein interference in an ADA [anti-drug antibody] assay." Spec. 4:28–30. It "also describes a number of modifications that can be made to variable domains in order to reduce or essentially avoid such protein interference." *Id.* at 5:4–5.

CLAIMED SUBJECT MATTER

Claim 51, reproduced below, illustrates the claimed subject matter:

> 51. A fusion protein comprising at least two immunoglobulin single variable domains (ISV), wherein one of the at least two ISVs is at the C-terminal end of the fusion protein, wherein the ISV at the C-terminal end of the fusion protein is a VHH, a humanized VHH, a VH, or a camelized VH that: does not bind to serum albumin; and has a C-terminal end of the sequence $VTVSS(X)_n$ (SEQ ID NO: 34), in which n is 1, 2, 3, 4, or 5, and in which each X is chosen from the group consisting of alanine (A), glycine (G), valine (V), leucine (L), and isoleucine (I), except with the proviso that when n is 3, each X is chosen from the group consisting of glycine (G), valine (V), leucine (L), and isoleucine (I), wherein the fusion protein does not comprise an ISV that binds IL-23 or other interleukins.

Appeal Br. 51 (Claims App.).

REFERENCES

The Examiner relies on the following references to reject the claims:

Name	Reference	Date
The '364 patent	US 11,319,364 B2	May 3, 2022
The '401 patent	US 11,603,401 B2	Mar. 14, 2023

Name	Reference	Date
The '307 patent	US 11,813,307 B2	Nov. 14, 2023
The '702 patent	US 11,932,702 B2	Mar. 19, 2024
The '797 patent	US 11,999,797 B2	June 4, 2024
The '308 patent	US 12,129,308 B2	Oct. 29, 2024
Silence	WO 2006/122825 A2	Nov. 23, 2006

REJECTIONS

The Examiner maintains the following rejections:[2]

1. Claims 51 and 54–56 are rejected on the ground of non-statutory double patenting ("NSDP") as being unpatentable over claims 1 and 5–13 of the '364 patent. Non-Final Act. 4.

2. Claims 51 and 54–56 are rejected on the ground of NSDP as being unpatentable over claims 1–2, 5, 11, and 12 of the '401 patent. *Id.* at 5.

3. Claims 51 and 54–56 are rejected on the ground of NSDP as being unpatentable over claims 1, 16, 24, and 25 of the '702 patent. *Id.* at 7.

4. Claims 51 and 54–56 are rejected on the ground of NSDP as being unpatentable over claims 1 and 6–8 of the '307 patent. *Id.*

5. Claims 51, 54, and 55 are rejected on the ground of NSDP as being unpatentable over claims 1, 9, 12, 14, and 15 of the '308 patent. *Id.*

6. Claim 56 is rejected on the ground of NSDP as being unpatentable over claims 1, 9–12, 14, and 15 of the '308 patent in view of Silence. *Id.* at 8.

7. Claims 51, 54, and 55 are rejected on the ground of NSDP as being unpatentable over claims 1 and 7 of the '797 patent. *Id.* at 9.

[2] The Examiner also provisionally rejected claims 51 and 54–56 on the ground of NSDP as being unpatentable over Application No. 14/128,681 and Application No. 18/145,989. Non-Final Act. 5, 6. Those rejections are withdrawn. Ans. 4.

8. Claims 56 is rejected on the ground of NSDP as being unpatentable over claims 1 and 7 of the '797 patent in view of Silence. *Id.*

OPINION

There are some undisputed facts in this case.

First, the instant application was filed on August 23, 2021. *See* Appeal Br. 1; Ans. 11. It is "a continuation of U.S. Application Serial No. 14/128,681, filed March 4, 2014, which is a national stage filing under 35 U.S.C. § 371 of international application PCT/EP2012/062251 ["the '251 application"], filed June 25, 2012." Spec. 1.

Second, the patent term filing date of the instant application is June 25, 2012, the filing date of the '251 application. Appeal Br. 10; Ans. 15.

Third, the '251 application was published as WO 2012/175741 on December 27, 2012. Appeal Br. 10.

Fourth, the publication date of the '251 application predates the patent term filing date of each reference patent (i.e, the '364 patent, the '401 patent, the '702 patent, the '307 patent, the '308 patent, and the '797 patent). Appeal Br. 10, 25, 34, 38, 42–43, 47; Ans. 15–16.

Fifth, "[t]he instant application does not share a common priority claim with any of the reference patents applied." Ans. 11.

Sixth, "the claims of the later-filed, now issued reference patents applied . . . were non-obvious over the instant disclosure and could not have been presented in the instant application." Appeal Br. 17; Ans. 11.

Seventh, "the instant application and each of the reference patents have a common assignee and/or a common inventor." Ans. 11; *see also id.*

at 14–15 ("[W]hile there are inventors in common between the instant application and the reference patents, the inventorship is not identical to any of the reference patents. While there is a common applicant between the instant application and the reference patents, the applicant is not identical" for the '364 patent, the '401 patent, the '307 patent, the '308 patent, and the '797 patent), 15 (the '702 patent "has an applicant identical to the instant application").

Qualification as NSDP References

The Examiner rejected the pending claims on the ground of NSDP over the '364 patent, the '401 patent, the '702 patent, the '307 patent, the '308 patent, and the '797 patent. Non-Final Act. 4–9. Appellant argues that none of the reference patents qualifies as an NSDP reference because each reference patent has a later patent term filing date compared to the instant application and expires later than any patent issuing from the instant application. Appeal Br. 8–14, 24–26, 33–35, 37–39, 41–44, 46–48.

Appellant relies on several Federal Circuit cases. Appeal Br. 8–12 (citing, affirming; *Allergan USA, Inc. v. MSN Lab'ys Priv. Ltd.*, 111 F.4th 1358 (Fed. Cir. 2024); *In re Cellect, LLC*, 81 F.4th 1216 (Fed. Cir. 2023); *Novartis AG v. Ezra Ventures LLC*, 909 F.3d 1367 (Fed. Cir. 2018); *Novartis Pharms. Corp. v. Breckenridge Pharm. Inc.*, 909 F.3d 1355 (Fed. Cir. 2018); *Gilead Sciences, Inc. v. Natco Pharma Ltd.*, 753 F.3d 1208 (Fed. Cir. 2014); *Acadia Pharms. Inc. v. Aurobindo Pharma Ltd.*, 706 F. Supp.3d 477 (D. Del. 2023), *aff'd*, 2025 WL 1618201 (Fed. Cir. June 9, 2025)).

Specifically, Appellant emphasizes that "the ***fundamental purpose*** of the NSDP doctrine 'is to prevent patentees from obtaining a *second* patent on a patentably indistinct invention to effectively extend the life of a *first*

patent to that subject matter.'" Appeal Br. 8 (quoting *Allergan*, 111 F.4th at 1369). Appellant also argues that "[i]f a later (actually) filed patent with the same patent term filing date is ineligible as an NSDP Reference – as decided by the district court (and affirmed by the Federal Circuit) in Acadia – a patent having a ***later patent term*** filing date . . . must surely be ineligible as an NSDP Reference." *Id.* at 10. Appellant further contends that "[a]n NSDP Reference must not have a later expiration date compared to the expiration date of a target claim." *Id.* at 11.

We acknowledge the case law relied on by Appellant but find they have limited application in this case. *Ezra Ventures* addresses the interaction between patent term extension ("PTE") under 35 U.S.C. § 156 and obviousness-type double patenting. 909 F.3d at 1373–74 ("[O]bviousness-type double patenting does not invalidate a validly obtained PTE."). *Cellect* grapples with the issue of how patent term adjustment ("PTA") affects an obviousness-type double patenting ("ODP") analysis. 81 F.4th at 1226–29 ("ODP for a patent that has received PTA, regardless whether or not a terminal disclaimer is required or has been filed, must be based on the expiration date of the patent after PTA has been added."). In *Breckenridge*, the court encountered a "particular situation" involving "an earlier-filed, earlier-issued, pre-URAA patent that expires after the later-filed, later-issued, post-URAA patent due to a change in statutory patent term law." 909 F.3d at 1366. The court used the pre-URAA patent's issuance date as the reference point for its obviousness-type double patenting analysis. *Id.* The *Gilead* court answered "a narrow question: Can a patent that issues after but expires before another patent qualify as a double patenting reference for that other patent?" and "h[e]ld that an earlier-expiring patent can qualify as

an obviousness-type double patenting reference for a later-expiring patent under the circumstances here." 753 F.3d at 1211–12, 1217. And the *Allergan* court answered a different but equally narrow question: "can a first-filed, first-issued, later-expiring claim be invalidated by a later-filed, later-issued, earlier-expiring reference claim having a common priority date?" and "h[e]ld that it cannot."111 F.4th at 1366. *Acadia* "is entirely controlled by" *Allergan* and "appl[ied] *Allergan*'s holding that 'a first-filed, first-issued, later-expiring claim cannot be invalidated by a later-filed, later-issued, earlier-expiring reference claim having a common priority date.'" 2025 WL 1618201 at *1 (quoting *Allergan*, 111 F.4th at 1369).

Despite Appellant's skillful case synthesis, the facts that underpinned the Federal Circuit's holdings in those cases are different from the facts in this case. A case that has a similar fact pattern to the instant case, but one not mentioned by Appellant, is *In re Fallaux*, 564 F.3d 1313, 1316 (Fed. Cir. 2009).

In *Fallaux*, the examiner rejected the pending claims in the Fallaux application for obviousness-type double patenting over certain claims of two reference patents. 564 F.3d at 1314–15. The Fallaux family of applications originated in a PCT application filed in June 1995. *Id.* at 1315. The first U.S. application in the family was filed in March 1997. *Id.* The Fallaux application was the fifth continuation application, claiming priority to the 1997 application. *Id.* The reference patents were filed in June 1998 and July 1999, respectively, and issued in July 2002 and January 2002, respectively. *Id.* Our predecessor, The Board of Patent Appeals and Interferences, affirmed the examiner's rejection. *Id.* at 1314–15. And the Federal Circuit affirmed the Board's decision. *Id.* at 1319.

Like this case, the reference patents in *Fallaux* have later patent term filing dates compared to the application under examination. Because they are post-URAA patents, they would expire twenty years from the patent term filing dates. *See Allergan*, 111 F.4th at 1367 (explaining that post-URAA, "a patent's term is now measured from its effective filing, or priority, date"). The application under examination was also filed post-URAA, and thus, any patent issued from the application would expire before the reference patents expired. Yet, the Federal Circuit upheld the Board's decision, affirming the NSDP rejection of the pending claims over the reference patents with later patent term filing dates and later expiration dates because "there is a second justification for obviousness-type double patenting—harassment by multiple assignees." *Fallaux*, 564 F.3d at 1318. As explained below, that justification remains relevant to the instant facts.

Fallaux remains good law. Indeed, the Federal Circuit cited *Fallaux* as recent as in *Allergan*. *See Allergan*, 111 F.4th at 1367 (citing *Fallaux*, 564 F.3d at 1318). Thus, under *Fallaux*, which is binding on us, we are not persuaded by Appellant's argument that the reference patents do not qualify as NSDP references because they each have a later patent term filing date compared to the instant application and expires later than any patent issuing from the instant application.

Patentable Distinctiveness

In each NSDP rejection, the Examiner found that the rejected claims, though not identical to, are not patentably distinct from, those asserted claims in the reference patents. Non-Final Act. 4, 5, 7–9. The Examiner provided reasoned explanation as to why the claims are not patentably

distinct. For example, for the rejection over the '401 patent, the Examiner found

> issued claim 5 as it depends from claim 1 is directed to a polypeptide that comprises at least two ISV s that specifically bind Aggrecan, wherein the at least two ISVs are the same. As recited in issued claim 2, the ISV can be SEQ ID NO: 117. SEQ ID NO: 117 ends in VTVSSA (i.e. where n =1 and X is alanine is in the instant claims). Issued claims 11-12 provide pharmaceutical compositions suggesting those of instant claim 56.

Id. at 5–6; *see also id.* at 4–5, 7–9 (explaining why the rejected claims are not patentably distinct from the asserted claims of the '364 patent, the '702 patent, the '307 patent, the '308 patent, and the '797 patent, respectively).

Appellant does not address the Examiner's analyses on patentable distinctiveness. *See generally* Appeal Br. Appellant, however, argues that "the two-way test, not the one-way test, should be used to assess patentable distinctiveness" (*id.* at 16, 27, 36, 40, 44, 49) "in any situation where the reference has a later patent term filing date, regardless of the issue date" (*id.* at 17).

Appellant acknowledges that "M.P.E.P. § 804 suggests that '[a] two-way test is to be applied only when the applicant could not have filed the claims in a single application ***and*** the Office is solely responsible for any delays.'" Appeal Br. 17. Appellant asserts that "the second prong of this test (i.e., demonstrating that 'the Office is solely responsible for any delays') is only relevant pre-URAA, and it is unfair and unreasonable post-URAA – especially in view of the statutes and regulations related to PTA, which were codified after enactment of the URAA." *Id.* According to Appellant, "the first [*sic*] prong of the two-way test is necessarily met

post-URAA as the Office is solely responsible for any delay that would result in a timewise extension (*i.e.*, PTA)." *Id.* Thus, Appellant contends that "only the first prong of the two-way test needs to be met for the two-way test to apply (i.e., demonstrating that the applicant could not have filed the claims in a single application), since the second prong is necessarily met." *Id.*

Appellant argues that it "could not have filed the instant claims and the reference claims in the instant application," and thus, met the first prong of the two-way test. *Id.* Ultimately, Appellant asserts that we should reverse the NSDP rejections under the two-way test because the claims of the reference patents were allowed over the disclosure of the instant application. *Id.*

We are not persuaded by Appellant's arguments. "In determining double patenting, a one-way test is normally applied, in which the examiner asks whether the application claims are obvious over the patent claims." *Fallaux*, 564 F.3d at 1316 (quotation marks omitted). "The two-way test is a narrow exception to the general rule of the one-way test. When applying the two-way test, the examiner also asks whether the patent claims are obvious over the application claims." *Id.* (internal citation and quotation marks omitted). "The two-way test is only appropriate in the unusual circumstance where, inter alia, the United States Patent and Trademark Office (PTO) is *solely* responsible for the delay in causing the second-filed application to issue prior to the first." *Id.* (quotation marks omitted).

Again, *Fallaux* is directly on point. In *Fallaux*, the examiner applied the one-way test to reject the Fallaux claims for NSDP. 564 F.3d at 1315. Dr. Fallaux argued that he was entitled to the two-way test before both the

Board and the Federal Circuit. *Id.* at 1315–16. Both the Board and the Federal Circuit rejected that argument. *Id.* at 1315–16, 1319. In *Fallaux*, the specification of the priority application would have supported the rejected claims. *Id.* at 1317. "Nonetheless, Dr. Fallaux elected to prosecute other applications and delay filing the Fallaux application until six years after the [priority] application was filed. During this six year period, the [reference] patents were filed for and issued." *Id.* In view of those facts, the Federal Circuit held that the PTO was not responsible for the delay and concluded that "[t]he Board correctly held that Dr. Fallaux cannot rely on the two-way test to overcome the rejection of the Fallaux claims for obviousness-type double patenting." *Id.* at 1319.

Similarly in this case, the priority application (the '251 application) was filed on June 25, 2012. Spec. 1. It entered the national stage with a U.S. utility application filed on March 4, 2014. *Id.* As the Examiner points out and Appellant does not dispute, "Appellant was not precluded from presenting and prosecuting the instant claims in the original application." Ans. 11.

Both 2012 and 2014 application dates precede the patent term filing dates of all reference patents, the earliest of which is May 13, 2015 for the '364 patent. Ans. 15. The instant application, however, was not filed until August 23, 2021, as a continuation of the 2014 national stage application. Spec. 1; Appeal Br. 1. In other words, Appellant chose to delay the filing of the instant application until more than nine years after

the '251 application was filed. During this nine-year period, the reference patents were filed and prosecuted.[3]

Thus, under *Fallaux*, a precedent binding on us, we agree with the Examiner that "the instant application is not one in which the Office was solely responsible for any delays," and "[a]s such, only a one-way determination of distinct[ive]ness is required." Ans. 11; *see Fallaux*, 564 F.3d at 1318; *In re Hubbell*, 709 F.3d 1140, 1150 (Fed. Cir. 2013) (holding the two-way test not applicable where "the PTO was not solely responsible for the delay" because the applicant could have presented the claims in one of its earlier applications in the family).

We find persuasive the Examiner's analyses of patentable distinctiveness and agree with the Examiner that, under the one-way test, the rejected claims are not patentably distinct from the asserted claims of all reference patents. *See* Non-Final Act. 4–9.

Equitable Considerations

Appellant argues that we should apply principles of equity to reverse the NSDP rejections. Appeal Br. 18–20.

We acknowledge Appellant's argument that "[b]ut for the fact that there are common inventors (though distinct overall inventive entities), a common applicant, and a common assignee, the Examiner could not have issued the NSDP rejection over [each reference] Patent [because each reference] Patent is not prior art due to its later effective filing date." Appeal Br. 23. But the Federal Circuit previously affirmed the Board's decision

[3] The '702 patent issued from an application filed on December 17, 2021, claiming priority to a provisional application filed on December 18, 2020. The '702 patent, codes 22, 60.

affirming NSDP rejections under similar circumstances. *See Hubbell*, 709 F.3d at 1143 n.2 ("[T]hough the [reference] patent issued first, it is not available as prior art under 35 U.S.C. §§ 102 or 103 against the claims in the . . . application [under examination.]").

Appellant contends that "allowing a patent (*e.g.*, the '364 Patent) to qualify as an NSDP Reference to a patent (or patent application) having an earlier patent term filing date (*e.g.*, the instant application) would open avenues for gamesmanship by others seeking to invalidate patent claims (or to block the issuance of a patent application)." Appeal Brief 19–20. We are not persuaded by this argument.

As discussed above, "[s]ome commonality of inventorship or (deemed) ownership must exist between two or more patents or applications before consideration can be given to the issue of double patenting." MPEP § 804. With common ownership, we are hard pressed to understand Appellant's alleged "gamesmanship by others." Where the NSDP is solely based on common inventorship, case law binding on us outweighs any equitable consideration. *See Fallaux*, 564 F.3d at 1315 (affirming NSDP rejection even though "[t]he reference patents for the double patenting rejection on appeal . . . are related to the Fallaux application only by way of a single common inventor").

Appellant also contends that "[a]llowing a patent having a later patent term filing date (*e.g.*, the '364 Patent) to qualify as an NSDP Reference to a patent (or patent application) having an earlier patent term filing date (*e.g.*, the instant application) would disincentivize patent owners from developing follow-on technologies." Appeal Br. 20. This is because, Appellant continues, "patenting these later technologies . . . would have the potential to

damage patent rights covering the earlier-developed generic technologies." *Id.* We are not persuaded by this argument either.

When facing an NSDP rejection over a patent, an applicant may timely file a terminal disclaimer to overcome the rejection. *Cellect*, 81 F.4th at 1226. As Appellant repeatedly emphasizes, a reference patent having a later patent term filing date generally would have a later expiration date. In our view, a terminal disclaimer over the later-expiring patent (covering the later technology) would not cut short the term of the patent covering the earlier-developed technology. Appellant has not clearly articulated any other damage to patent rights covering the earlier-developed technologies.

Appellant further asserts that "double patenting issues would potentially restrict the patent owners' ability to alienate their patent rights covering the later technologies (*e.g.*, because of common ownership requirements for terminal disclaimers)." Appeal Br. 20. Appellant is reminded that although the patent covering the later technology has issued, the instant application is still under examination. To overcome the NSDP rejection, Appellant is free to amend the pending claims so that they are patentably distinct from the issued claims. That would obviate the need to file a terminal disclaimer. To the extent Appellant is unwilling to do so, a timely filed terminal disclaimer complying with 37 C.F.R. § 1.321(c), including satisfying the common ownership requirement, is the quid pro quo for obtaining claims patentably indistinct from claims that are already issued.

Indeed, the common ownership requirement in a terminal disclaimer serves the purpose to prevent the possibility of multiple suits against an accused infringer by different assignees of patents claiming patentably

indistinct variations of the same invention. *In re Van Ornum*, 686 F.2d 937, 944–48 (CCPA 1982). Appellant argues that *Van Ornum* and its progeny "were wrongly decided to the extent they may have relied on or otherwise endorsed an anti-harassment rationale as the sole basis for upholding an NSDP rejection." Appeal Br. 21. The Board is not the proper venue to address this argument. Because Appellant points to no legal authority showing that *Van Ornum* has been overruled, *Van Ornum* remains binding on us.

Moreover, Appellant emphasizes that "the ***fundamental purpose*** of the NSDP doctrine 'is to prevent patentees from obtaining a *second* patent on a patentably indistinct invention to effectively extend the life of a *first* patent to that subject matter.'" Appeal Br. 8 (quoting *Allergan*, 111 F.4th at 1369). But, as the Federal Circuit recognized, "the unjustified patent term extension justification for obviousness-type double patenting has limited force in . . . many double patenting rejections today, in no small part because of the change in the Patent Act from a patent term of seventeen years from issuance to a term of twenty years from filing." *Fallaux*, 564 F.3d at 1318. The court confirmed this understanding in *Allergan*. 111 F.4th at 1367 ("[P]ost-URAA, there is little risk of an unjustified extension of term subject to ODP because all patents to an invention that share a priority date are expected to expire on the same day."). Although the court "recognized that the doctrine of obviousness-type double patenting is less significant in post-URAA patent disputes," it "also recognized its continued importance." *Abbvie Inc. v. Mathilda & Terence Kennedy Inst. of Rheumatology Tr.*, 764 F.3d 1366, 1373 (Fed. Cir. 2014).

In *Fallaux*, the Federal Circuit relied on a different "justification for obviousness-type double patenting—harassment by multiple assignees" in upholding the Board's decision affirming the NSDP rejection. 564 F.3d at 1319. The court later applied the multiple-assignee-harassment rationale again. *See Hubbell*, 709 F.3d 1147–48 ("Because it is undisputed that an infringer of the [reference] patent would also infringe the . . . application [under examination], the multiple assignee harassment justification adopted in *Van Ornum* and reaffirmed in *Fallaux* applies here."); *see also Cellect*, 81 F.4th at 1230 (concluding "the Board did not err in determining that a risk of separate ownership existed and, even in the absence of separate ownership, that a terminal disclaimer would have been required to ensure common ownership" and stating "[w]hile [the patent owner] has not engaged in actions that resulted in divided ownership in the past, and it has promised that it will not do so in the future, neither fact suffices to abrogate the potential future risk of multiple owners or assignees").

Under the Federal Circuit precedent, the Examiner did not err in stating that under the guidance under MPEP § 804.02(VI), "it is appropriate for an examiner to require a terminal disclaimer that will address the risk of separate ownership even where it is possible that there would be no unjustified timewise extension of the right to exclude." Ans. 18. In this case, Appellant's argument protesting "double patenting issues would potentially restrict the patent owners' ability to alienate their patent rights" highlights the risk of separate ownership. *See* Appeal Br. 20.

Ex Parte Baurin

Appellant argues that

> [t]he material facts of the instant case are the same as those at issue in Ex Parte Baurin et al., Appeal 2024-002920 (P.T.A.B. Nov. 6, 2024), in which the Board held that the asserted references ***were not*** proper NSDP References and overturned the asserted NSDP rejections in view of these references.

Appeal Br. 14, 26, 35, 39, 44, 48.

Ex Parte Baurin is not currently a precedential Board decision and is therefore not binding on us. Moreover, as the Examiner points out, in that case,

> a request for rehearing was made by the examiner under MPEP 1214.04 on 3 January 2025. Appellant responded on 29 January 2025. These materials are publicly available through the USPTO Patent Center. To date, the request for rehearing has not been granted or denied. No decision has been made on the merits of the requested rehearing.

Ans. 3. Thus, we do not consider the Board's decision in Appeal 2024-002920.

CONCLUSION

The Examiner's rejections are AFFIRMED.

Appeal 2026-000193
Application 17/409,019

DECISION SUMMARY

The following table summarizes our decision:

Claim(s) Rejected	35 U.S.C. §	Reference(s)/ Basis	Affirmed	Reversed
51, 54–56		Non-statutory Double Patenting US 11,319,364	51, 54–56	
51, 54–56		Non-statutory Double Patenting US 11,603,401	51, 54–56	
51, 54–56		Non-statutory Double Patenting US 11,932,702	51, 54–56	
51, 54–56		Non-statutory Double Patenting US 11,813,307	51, 54–56	
51, 54, 55		Non-statutory Double Patenting US 12,129,308	51, 54, 55	
56		Non-statutory Double Patenting US 12,129,308, Silence	56	
51, 54, 55		Non-statutory Double Patenting US 11,999,797	51, 54, 55	
56		Non-statutory Double Patenting US 11,999,797, Silence	56	
Overall Outcome			51, 54–56	

TIME PERIOD FOR RESPONSE

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a). *See* 37 C.F.R. § 1.136(a)(1)(iv).

AFFIRMED

TECHNIQUES FOR PREDICTING, DETECTING AND REDUCING ASPECIFIC PROTEIN INTERFERENCE IN ASSAYS INVOLVING IMMUNOGLOBULIN SINGLE VARIABLE DOMAINS

RELATED APPLICATIONS

This application is a continuation of U.S. Application Serial No. 14/128,681, filed March 4, 2014, which is a national stage filing under 35 U.S.C. § 371 of international application PCT/EP2012/062251, filed June 25, 2012, which was published under PCT Article 21(2) in English, which claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application number 61/500,360, filed June 23, 2011, U.S. provisional application number 61/500,464, filed June 23, 2011, and U.S. provisional application number 61/541,368, filed September 30, 2011; PCT/EP2012/062251 also claims the benefit under 35 U.S.C. § 120 of international application PCT/EP2011/067132, filed September 30, 2011, U.S. application number 13/435,567, filed March 30, 2012 and now issued as U.S. Patent 8,703,135, and international application PCT/EP2012/061304, filed June 14, 2012. The disclosures of all of the foregoing applications are incorporated by reference herein in their entireties.

REFERENCE TO SEQUENCE LISTING SUBMITTED AS A TEXT FILED VIA EFS-WEB

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 20, 2021, is named A084870142US12-SEQ-CRP and is 125,184 bytes in size.

The present invention relates to the field of immunoglobulin single variable domains.

An immunoglobulin single variable domain or "ISV" is generally defined herein as an amino acid sequence that:

- comprises an immunoglobulin fold or that, under suitable conditions (such as physiological conditions) is capable of forming an immunoglobulin fold (*i.e.,* by folding),

i.e., so as to form an immunoglobulin variable domain (such as, for example, a VH, VL or VHH domain);

and that

- forms (or under such suitable conditions is capable of forming) an immunoglobulin variable domain that comprises a functional antigen binding site (in the sense that it does not require an interaction with another immunoglobulin variable domain (such as a VH-VL interaction) to form a functional antigen binding site).

Some examples of immunoglobulin single variable domains that are currently known in the art are VHH's and/or (other) Nanobodies, dAb's and (single) domain antibodies. Of these, as of the date of filing of the present application, various Nanobodies are in phase I and phase II clinical trials. This makes it important to have available reliable assays for analyzing biological samples from people that are treated with ISV's (such as clinical trial subjects and, after such ISV's reach the market, patients that are treated with such ISV's).

This is not only important for regulatory purposes, but also for the treatment of patients with biological drugs, because the clinicians that prescribe the treatment would also like to have available reliable assays to monitor various aspects of the treatment.

For example, in the clinical development of biological drug molecules, it is important to assess their immunogenic potential, and in particular the degree to which they can elicit so-called "anti-drug antibodies" or "ADA's". This is determined using so-called "anti-drug antibody" or "ADA (immuno)assays" (see for example the review by Shankar et al., Journal of Pharmaceutical and Biomedical Analysis, 48 (2008), 1267-1281; as well as Mire-Sluis et al., J. Immunol. Meth. 289 (2004), 1-16; Peng et al., Journal of Pharmaceutical and Biomedical Analysis, 54, (2011), 629-635; and Loyet et al., J. Immunol. Meth. 345 (2009), 17-28. Such ADA assays and methods for performing them are standard knowledge in the field of pharmacology and they are routinely used during the clinical development of biological drug products (as well as being required by various regulatory agencies around the world).

For example, as described on pages 3 and 4 of the article by Mire-Sluis and as for example also exemplified schematically in the Figures of the article by Peng, a number of different ADA assay formats are known, such as "ELISA-bridging format", "ELISA-Direct Format", "Indirect Format", Radio Immuno-precipitation Assay (RIP), "Surface Plasmon

Resonance" and "Electrochemiluminescence-Bridging Format". Other formats for performing ADA immunoassays will be clear to the skilled person.

The skilled person will also be familiar with a number of different commercially available technology platforms that have been shown to be suitable for setting up and performing ADA assays. These include but are not limited to the MSD platform (Mesoscale), Gyrolab (Gyros) and the octet platform (Fortebio).

Some non-limiting examples of ADA assay formats are also schematically shown in Figures 1A to 1C.

Generally, it should be noted that in such ADA assays for detecting or measuring ADA's against an ISV, the ISV is used as the "analytical agent" (*i.e.,* as the compound used to detect whether any ADA's are present in the sample that is tested), and the ADA's are the "antigen" (*i.e.,* the compound to be detected in the sample that is tested). Thus, in these assays, the ISV will usually/often be bound to the carrier (such as the ELISA plate), whereas the ADA's (if any) will be present in the sample that is subjected to the assay.

To better understand the invention described herein, it should already be noted that - by contrast - in the methods that are used herein to predict whether an ISV will give rise to protein interference, the ISV will usually be used as the "antigen" (*i.e.,* as the compound to be detected), and an antibody (which is as further described herein) is used as the "analytical agent" (*i.e.,* as a means to detect whether a given ISV binds or not, respectively; and thus has a high or increased risk of giving rise to protein interference or not, respectively). Thus, in this method according to the invention, the antibody used as analytical agent (which is also referred to herein as the "*analytical antibody*") will usually be bound to the carrier (*i.e.,* to the ELISA plate) and the ISV will be (present in) the sample to be tested. However, it should generally be noted that the invention is not limited to assays in which the "analytical antibody" is bound to the carrier. For example, in an alternative way of performing an assay according to the invention (As shown for instance in Figure 1 and described in the Examples), the analytical antibody is instead used as a bridging agent and thus will be in solution rather than bound to the plate (although it is indirectly bound to the plate via the ISV that is coated on the plate). However, also in the specific bridging assay described in the Examples (which is a competitive assay) the analytical antibody is still used as the analytical agent (*i.e.,* to determine whether the ISV of interest binds or not, respectively; and thus has a high or increased risk of giving rise to protein interference or not, respectively). It is also

envisaged that, based on the further disclosure herein, the skilled person will be able to design other assay formats in which the analytical antibody can be used as an analytical agent in order to determine whether a given ISV can bind or not, respectively; and thus has a high or increased risk of giving rise to protein interference or not).

As a result of research into single chain Fv's or "ScFv's" (which are constructs that contain immunoglobulin single variable domains that, similar to ISV's, are not associated with constant domains), it has been described in the art that the C-terminus of an immunoglobulin variable domain forms a hydrophobic patch that in an antibody is buried in the interface between the variable domain and the constant domain but that becomes solvent-exposed when the variable domain is not associated with a constant domain (Nieba et al., Protein Engineering, 10, 435-444 (1997)). It has also been described that the exposed C-terminus may form B-cell epitopes which can give rise to and/or interact with (emerging and/or pre-existing) anti-drug antibodies (WO 11/07586), the presence of which can then be determined using the ADA assays referred to above. For this reason, it has been proposed to make mutations to some of the amino acid residues that form part of the C-terminus of the variable domains to reduce said hydrophobicity and/or to remove said epitopes. For example, Nieba et al. suggest to mutate positions 11, 14, 41, 84, 87 and/or 89 of a VH region (numbering according to Kabat), whereas in WO 11/07586 it is suggested to mutate positions 99, 101 and/or 148 (AHo numbering) of a VL domain or positions 12, 97, 98, 99, 103 and/or 144 of a VH domain (again AHo numbering - these positions correspond to positions 11, 83, 84, 85, 89 and 103 according to Kabat).

However, neither of these references recognizes that certain proteins present in the blood or serum of a subject can interfere with ADA assays involving ISV's, and because of this these references are not directed to (nor offer a solution for) the problem of how to avoid aspecific protein interference in such ADA assays so as to allow the ADA assay to be used to determine the true presence/extent of (arising or pre-existing) anti-drug antibodies in the sample to be tested.

By contrast, the present invention provides methods and assays that easily allow the skilled person to predict whether an immunoglobulin single variable domain will or will not have a tendency to undergo aspecific protein interference in an ADA assay. The methods and assays described herein also allow the skilled person, when it is found that a variable domain may have a tendency or risk to undergo such protein interference in an ADA assay, to easily

test (proposed) modifications to a variable domain in order to predict whether any such (proposed) modifications will reduce or essentially completely avoid such protein interference.

The present invention also describes a number of modifications that can be made to variable domains in order to reduce or essentially avoid such protein interference. According to one non-limiting aspect, this modification involves adding a limited number (as further described herein) of amino acid residues (as further described herein) to the C-terminal end of the variable domain. Surprisingly, it has been found that, for a number of different variable domains or constructs based thereon, even adding a single amino acid residue to the C-terminal end (such as a single alanine residue) can substantially or even essentially completely remove the problem of protein interference in ADA assays, even though adding one such amino acid is by itself is not sufficient to "cover" or "bury" the hydrophobic patch that according to Nieba et al. is present at the C-terminus of an ISV. Similarly, but without wishing to limit the invention in any way or to any mechanism or explanation, is also assumed that adding one such amino acid would not be sufficient to "cover " or "bury" any B-cell epitopes that according to WO 11/07586 may be present at the C-terminus of a variable domain It should also be noted that, although according to this specific aspect of the present invention, adding a limited number or even a single amino acid at the C-terminus of the variable domain (i.e. without making any substitutions within the C-terminal region itself, as proposed by Nieba et al and WO 11/07586) may - and in many cases will - significantly reduce or even essentially remove the problem of aspecific protein interference, it is also within the scope of this aspect of the invention that such additions to the C-terminal end are combined with mutations in the C-terminal region. In this respect, however, it should also be noted that the invention is not particularly limited as to the rationale behind making such mutations. For example, it is well known to make mutations to amino acid residues within the C-terminus (including at those positions that are explicitly referred to by Nieba et al. and in WO 11/07586) in order to humanize a variable domain (including, without limitation, a V_{HH} domain) or in order to "camelize" a V_H domain (reference is for example made to WO 08/020079 and some of the other applications by Ablynx N.V. referred to herein).

It is envisaged that the methods, assays and modifications taught herein can be applied to any variable domain that is not linked to or otherwise associated with a constant domain (or with another group or peptide moiety that functions to "shield", cover or "bury" the C-

terminal region of the variable domain) and more generally to any variable domain that has a C-terminal regions that is solvent-exposed. However, according to one preferred, but non-limiting aspect of the invention, the methods, assays and modifications may in particular be applied to heavy chain variable domains (V_H domains), and according to one specific aspect of the invention to V_{HH} domains.

It is also envisaged that the methods, assays and modifications described herein can be suitably applied to protein constructs that contain one or more variable domains, and in particular to such constructs in which a variable domain forms the C-terminal part of the construct or, in the case of the methods and assays described herein, in which the C-terminal region of a variable domain is otherwise solvent-exposed. Again, according to one preferred, but non-limiting aspect of the invention, the methods, assays and modifications are applied to constructs in which a V_H domain (and in particular a V_{HH} domain) forms the C-terminal part of the construct or, in case of the methods and assays of the invention, is otherwise solvent-exposed.

Some non-limiting examples of such constructs are multivalent, multispecific (such as bispecific) or multiparatopic (such as biparatopic) constructs that contain two or more ISV's linked directly or via one or more suitable linkers (with again, according to one specific aspect, a V_H or V_{HH} domain) forming the C-terminal part of such a construct. For example, and without limitation, such a construct may be entirely comprised of V_H domains, and in particular of Nanobodies (i.e. V_{HH} domains, humanized V_{HH} domains or camelized V_H domains), again linked directly or via one or more suitable linkers. For some non-limiting examples of such constructs and a general teaching on how such constructs can be made (in particular based on Nanobodies) reference is for example made to Conrath et al., JBC 276, 10(9), 7346 (2001) as well as to the review article by Muyldermans. Reviews in Mol. Biotechnol., 74: 27 (2001).

However, it is for example also envisaged that the invention can be applied to other constructs which have a solvent-exposed variable domain and in particular have a variable domain at their C-terminus, such as for example single chain Fv's, and in particular ScFv's that have their heavy chain variable domain at the C-terminus.

In the present specification and claims, terms like “ISV”, “analytical agent” and “protein interference” have the meaning as further defined herein.

In particular, an ISV as described herein may in particular either be a Nanobody or an(other) ISV (i.e. other than a Nanobody) that is a VH domain or that comprises a VH domain; and is preferably a Nanobody.

Also, any protein or polypeptide that comprises an ISV (such as an ISV-based drug) preferably has said (or at least one) such ISV at its C-terminal end. Again, said ISV may in particular either be a Nanobody or an(other) ISV (i.e. other than a Nanobody) that is a VH domain or that comprises a VH domain; and is preferably a Nanobody.

The invention described herein is in particular intended and suitable to be applied to ISVs that comprise, are based on and/or have been derived from heavy chain variable domains, such as VH domains (including human VH domains) and Nanobodies such as VHH domains (including humanized and sequence optimized VHH domains) or camelized VH domains. These may be synthetic (for example, obtained starting from a synthetic library and/or based on a fixed framework regions), semi-synthetic (for example, humanized, camelized or sequence-optimized, or obtained by affinity maturation or CDR grafting, starting from a natural VH or VHH domain) or fully naturally occurring VH or VHH domains. The invention will therefore be further described herein with reference to ISV's that are, are based on and/or have been derived from VH or VHH domains.

In establishing the present invention, it has been found that in some assays (such as, for example, in ADA immunoassays) that are used for analyzing biological samples (such as blood samples including whole blood, serum and plasma, ocular fluid, bronchoalveolar fluid/BALF, cerebrospinal fluid or other samples of biological fluids) protein interference may occur, and that such protein interference may give rise to an aspecific signal in some of these assays and/or in some of these samples. It has also been found that such protein interference may occur not only in samples that were obtained from subjects that have been treated with ISV's (and in particular with Nanobodies; or with proteins, polypeptides or other biological drugs that comprise at least one such ISV or Nanobody) and/or to whom the same have been administered (such as patients or clinical trial subjects), but also in samples from subjects that have never received an ISV (indicating that such interference is likely due to an aspecific protein-protein interaction with pre-existing proteins rather than any emerging ADA's).

Although it has been found that such protein interference and/or such a signal in such assays is not associated with any change or reduction in pharmacological properties (such as

pharmacokinetic/PK or pharmacodynamic/PD properties) of the ISV's, it would be desirable to have techniques available for predicting, detecting, reducing and/or if possible avoiding such aspecific protein interference. This is the general objective of the present invention.

In particular, the invention provides, and in certain specific but non-limiting aspects relates to:

- assays that can be used to predict whether a given ISV will be subject to such protein interference and/or give rise to such an (aspecific) signal in such an assay (such as for example in an ADA immunoassay). Such predictive assays could for example be used to test whether a given ISV could have a tendency to give rise to such protein interference and/or such a signal; to select ISV's that are not or less prone to such protein interference or to giving such a signal; as an assay or test that can be used to test whether certain modification(s) to an ISV will (fully or partially) reduce its tendency to give rise to such interference or such a signal; and/or as an assay or test that can be used to guide modification or improvement of an ISV so as to reduce its tendency to give rise to such protein interference or signal;
- methods for modifying and/or improving ISV's to as to remove or reduce their tendency to give rise to such protein interference or such a signal;
- modifications that can be introduced into an ISV that remove or reduce its tendency to give rise to such protein interference or such a signal;
- ISV's that have been specifically selected (for example, using the assay(s) described herein) to have no or low(er)/reduced tendency to give rise to such protein interference or such a signal;
- modified and/or improved ISV's that have no or a low(er)/reduced tendency to give rise to such protein interference or such a signal.

For example, in a first non-limiting aspect, the invention relates to a method that can be used to predict whether a given ISV or Nanobody (or ISV-based or Nanobody-based drug) will give rise to (or has an high or increased risk of giving rise to) protein interference in an immunoassay (*i.e.,* after it has been administered to a subject, a sample of a biological fluid has been obtained from said subject, and said sample is subjected to an immunoassay as further described herein), said method comprising performing an immunoassay that at least comprises the steps of:

(i) contacting said ISV or Nanobody (or ISV-based or Nanobody-based drug) with an antibody that has been obtained from a human subject and that has been selected, generated and/or isolated based on its ability to recognize and/or bind to the C-terminal end of an ISV or Nanobody (the "*analytical antibody*"); and

(ii) determining whether said ISV or Nanobody (or ISV-based or Nanobody-based drug) is bound by said antibody in said immunoassay.

In this method, when the ISV, Nanobody, ISV-based drug or Nanobody-based drug is bound by said analytical antibody, it can be expected that said ISV, Nanobody, ISV-based drug or Nanobody-based drug will give rise to (or has a high or increased risk of giving rise to) such protein interference (as further defined herein). Based on this, for example, said ISV, Nanobody, ISV-based drug or Nanobody-based drug may be modified or improved so as to reduce or remove its tendency to give rise to such protein interference (which may again be determined using the assay above), and some strategies that can be used to modify said ISV, Nanobody, ISV-based drug or Nanobody-based drug will be described herein (and for example include attaching a small number of amino acid residues to the C-terminal end and/or introducing one or more specific amino acid substitutions).

Thus, generally, the invention makes available to the skilled person assays and methods/techniques that can be used to predict the tendency of an ISV, Nanobody, ISV-based drug or Nanobody-based drug to give rise to protein interference and/or as a tool to improve ISVs so as to reduce or avoid their tendency to give rise to protein interference. In doing so, the invention also provides the skilled person with means to select ISV's, Nanobodies, ISV-based drugs or Nanobody-based drugs based on their low or reduced ability (or the absence of any ability) to give rise to protein interference. Thus, the invention provides the skilled person with an important assay and tool that can be used in the optimization and development of ISV's, Nanobodies, ISV-based drugs or Nanobody-based drugs.

Also, as further described herein, the invention teaches the skilled person a number of ways in which an ISV, Nanobody, ISV-based drug or Nanobody-based drug can be modified or improved so as to reduce or avoid their tendency to give rise to protein interference. Thus, the invention also generally makes available modified and/or improved ISV's, Nanobodies, ISV-based drugs or Nanobody-based drugs with a reduced, low or without any tendency to give rise to protein interference.

As further described herein, the invention can in particular be used to predict whether a given ISV or Nanobody (or ISV-based or Nanobody-based drug) will give rise to protein interference (as further described herein) in an immunoassay, and more in particular in an ADA assay. Said ADA assay may for example be an ADA assay for detecting or measuring ADA's against ISV's generally, and may in particular be an ADA assay for detecting or measuring ADA's against the ISV used in steps (i) and (ii) above.

Again, as mentioned herein, an ISV as described herein may in particular either be a Nanobody or an(other) ISV (i.e. other than a Nanobody) that is a VH domain or that comprises a VH domain; and is preferably a Nanobody.

Also, any protein or polypeptide that comprises an ISV (such as an ISV-based drug) preferably has said (or at least one) such ISV at its C-terminal end. Again, said ISV may in particular either be a Nanobody or an(other) ISV (i.e. other than a Nanobody) that is a VH domain or that comprises a VH domain; and is preferably a Nanobody.

The sample that is tested in said immunoassay or ADA assay is also referred to herein as the "*test sample*" or "*assay sample*". To avoid confusion, such as "*test sample*" or "*assay sample*" should not be confused with the biological sample that is used herein as a starting material for obtaining the (polyclonal or monoclonal) "analytical antibody" used in the invention.

In one particular preferred but non-limiting aspect, the invention can be used to predict whether a given ISV or Nanobody (or ISV-based or Nanobody-based drug) will give rise to protein interference (as further described herein) in an immunoassay (and in particular, in an ADA assay) that involves the use of such an ISV. Again, said ADA assay may for example be an ADA assay for detecting or measuring ADA's against ISV's generally, and may in particular be an ADA assay for detecting or measuring ADA's against the ISV used in steps (i) and (ii) above.

In an even more particular but non-limiting aspect, the invention can be used to predict whether a given ISV or Nanobody (or ISV-based or Nanobody-based drug) will give rise to protein interference (as further described herein) in an immunoassay (and in particular, in an ADA assay) that is intended to determine or measure whether the sample contains any ADA's against the ISV. Again, for example, such an immunoassay may be one of the known types of ADA assay (for which reference is for example made to the prior art on ADA assays cited herein) that is performed to determine or measure whether any ADA's against said ISV

are present in the "test sample", wherein said test sample is a sample of biological fluid (as described herein) that is obtained from a subject to which said ISV has been administered (as further described herein).

As further described herein, in all these aspects (and the further aspects of the invention described herein), the invention can also be used to select ISV's that are not or less prone to such protein interference in such immunoassays or ADA assays; as an assay or test that can be used to test whether certain modification(s) to an ISV will (fully or partially) reduce its tendency to give rise to such interference in such immunoassays or ADA assays; and/or as an assay or test that can be used to guide modification or improvement of an ISV so as to reduce its tendency to give rise to such protein interference in such immunoassays or ADA assays.

Other aspects, embodiments, advantages and applications of the invention will become clear from the further description herein.

In the present specification, whenever the term "ISV" is used, it should be understood that:

- such an ISV is preferably a Nanobody, in which the term "Nanobody" is generally as defined in or WO 08/020079 or WO 09/138519, and thus in a specific aspect generally denotes a VHH, a humanized VHH or a camelized VH (such as a camelized human VH) or generally a sequence optimized VHH (such as e.g. optimized for chemical stability and/or solubility, maximum overlap with known human framework regions and maximum expression). It is noted that the terms Nanobody or Nanobodies are registered trademarks of Ablynx N.V. and thus may also be referred to as Nanobody® and/or Nanobodies®);
- the term "ISV" in its broadest sense also includes "ISV-based biologicals" and, when the ISV is a Nanobody, "Nanobody-based biologicals". An "ISV-based biological" is defined herein as a protein, polypeptide or other biological drug that comprises or essentially consist of at least one (such as one, two or three) ISV's. Similarly, a "Nanobody-based biological" is defined as a protein, polypeptide or other biological drug that comprises or essentially consist of at least one (such as one, two or three) Nanobodies. As with the term "ISV", whenever the term "ISV-based biological" is used, it should be understood that such an ISV-based biological is preferably a Nanobody-based biological. Within the context of the present invention, both an "ISV-based biological" and a "Nanobody-based

biological" may for example be a monovalent, bivalent (or multivalent), bispecific (or multispecific), and biparatopic (or "multiparatopic) ISV construct or Nanobody construct, respectively. Also, any ISV-based or Nanobody-based biological may for example, in addition to the one or more (such as one, two or three) ISV's or Nanobodies, optionally further comprise one or more (such as one or two) other further therapeutic moieties and/or one or more (such as one or two) other moieties that influence the pharmacokinetic or pharmacodynamic properties of the ISV-based or Nanobody-based biological (such as its half-life). Suitable examples of such further therapeutic or other moieties will be clear to the skilled person, and for example generally can include any therapeutically active protein, polypeptide or other binding domain or binding unit, as well as for example modifications such as those described on pages 149 to 152 of WO 09/138159. An ISV-based biological or Nanobody-based biological is preferably a therapeutic or intended for use as a therapeutic (which includes prophylaxis and diagnosis) and for this purpose preferably contains at least one ISV against a therapeutically relevant target (such as for example RANK-L, vWF, IgE, RSV, CXCR4, IL-23 or other interleukins, etc.). For some specific but non-limiting examples of such ISV-based or Nanobody-based biologicals, reference is for example made to the various applications by Ablynx N.V. (such as for example and without limitation WO 2004/062551, WO 2006/122825, WO 2008/020079 and WO 2009/068627), as well as for example (and without limitation) to applications such as WO 06/038027, WO 06/059108, WO 07/063308, WO 07/063311, WO 07/066016 and WO 07/085814.Also, in the present specification, unless explicitly mentioned otherwise herein, all terms mentioned herein have the meaning given in WO 09/138519 (or in the prior art cited in WO 09/138519) or WO 08/020079 (or in the prior art cited in WO 08/020079). Also, where a method or technique is not specifically described herein, it can be performed as described in WO 09/138519 (or in the prior art cited in WO 09/138519) or WO 08/020079 (or in the prior art cited in WO 08/020079).

In particular, the following terms have the same meaning as given on, and/or where applicable can be determined in the manner described in, pages 62-75 of WO 09/138519: "*agonist*", "*antagonist*", "*inverse agonist*", "*non-polar, uncharged amino acid residue*", "*polar uncharged amino acid residue*", "*polar, charged amino acid residue*", "*sequence identity*", "*exactly the same*" and "*amino acid difference*" (when referring to a sequence comparison of two amino acid sequences), "*(in) essentially isolated (form)*", "*domain*",

"*binding domain*", "*antigenic determinant*" ,"*epitope*", "*against*" or "*directed against*" (an antigen),"*specificity*" and "*half-life*". In addition, the terms "*modulating*" and "*to modulate*", "*interaction site*", "*specific for*", "*cross-block*", "*cross-blocked*" and "*cross-blocking*" and "*essentially independent of the pH*" are as defined on (and/or can be determined as described on) pages 74-79 of WO 10/130832 of applicant. Also, when referring to a construct, compound, protein or polypeptide of the invention, terms like "*monovalent*", "*bivalent*" (or "*multivalent*"), "*bispecific*" (or "*multispecific*"), and "*biparatopic*" (or "*multiparatopic*") may have the meaning given in WO 09/138.519, WO 10/130832 or WO 08/020079.

The term "half-life" as used herein relation to an ISV, Nanobody, ISV-based biological, Nanobody-based biological or any other amino acid sequence, compound or polypeptide can generally be defined as described in paragraph o) on page 57 of WO 08/020079 and as mentioned therein refers to the time taken for the serum concentration of the amino acid sequence, compound or polypeptide to be reduced by 50%, in vivo, for example due to degradation of the sequence or compound and/or clearance or sequestration of the sequence or compound by natural mechanisms. The in vivo half-life of an amino acid sequence, compound or polypeptide of the invention can be determined in any manner known per se, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally be as described in paragraph o) on page 57 of WO 08/020079. As also mentioned in paragraph o) on page 57 of WO 08/020079, the half-life can be expressed using parameters such as the t1/2-alpha, t1/2-beta and the area under the curve (AUC). In this respect it should be noted that the term "half-life" as used herein in particular refers to the t1/2-beta or terminal half-life (in which the t1/2-alpha and/or the AUC or both may be kept out of considerations). Reference is for example made to the Experimental Part below, as well as to the standard handbooks, such as Kenneth, A et al: Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and Peters et al, Pharmacokinete analysis: A Practical Approach (1996). Reference is also made to "Pharmacokinetics", M Gibaldi & D Perron, published by Marcel Dekker, 2nd Rev. edition (1982). Similarly, the terms "increase in half-life" or "increased half-life" as also as defined in paragraph o) on page 57 of WO 08/020079 and in particular refer to an increase in the t1/2-beta, either with or without an increase in the t1/2-alpha and/or the AUC or both.

When a term is not specifically defined herein, it has its usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the standard

handbooks, such as Sambrook et al, "Molecular Cloning: A Laboratory Manual" (2nd.Ed.), Vols. 1-3, Cold Spring Harbor Laboratory Press (1989); F. Ausubel et al, eds., "Current protocols in molecular biology", Green Publishing and Wiley Interscience, New York (1987); Lewin, "Genes II", John Wiley & Sons, New York, N.Y., (1985); Old et al., "Principles of Gene Manipulation: An Introduction to Genetic Engineering", 2nd edition, University of California Press, Berkeley, CA (1981); Roitt et al., "Immunology" (6th. Ed.), Mosby/Elsevier, Edinburgh (2001); Roitt et al., Roitt's Essential Immunology, 10th Ed. Blackwell Publishing, UK (2001); and Janeway et al., "Immunobiology" (6th Ed.), Garland Science Publishing/Churchill Livingstone, New York (2005), as well as to the general background art cited herein.

Also, herein, the amino acid residues of a Nanobody are numbered according to the general numbering for VH domains given by Kabat et al. ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, MD, Publication No. 91), as applied to VHH domains from Camelids in the article of Riechmann and Muyldermans, J. Immunol. Methods 2000 Jun 23; 240 (1-2): 185-195; or referred to herein. According to this numbering, FR1 of a Nanobody comprises the amino acid residues at positions 1-30, CDR1 of a Nanobody comprises the amino acid residues at positions 31-35, FR2 of a Nanobody comprises the amino acids at positions 36-49, CDR2 of a Nanobody comprises the amino acid residues at positions 50-65, FR3 of a Nanobody comprises the amino acid residues at positions 66-94, CDR3 of a Nanobody comprises the amino acid residues at positions 95-102, and FR4 of a Nanobody comprises the amino acid residues at positions 103-113. [In this respect, it should be noted that - as is well known in the art for VH domains and for VHH domains - the total number of amino acid residues in each of the CDR's may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering). This means that, generally, the numbering according to Kabat may or may not correspond to the actual numbering of the amino acid residues in the actual sequence. Generally, however, it can be said that, according to the numbering of Kabat and irrespective of the number of amino acid residues in the CDR's, position 1 according to the Kabat numbering corresponds to the start of FR1 and vice versa, position 36 according to the Kabat numbering corresponds to the start

of FR2 and vice versa, position 66 according to the Kabat numbering corresponds to the start of FR3 and vice versa, and position 103 according to the Kabat numbering corresponds to the start of FR4 and vice versa.].

Alternative methods for numbering the amino acid residues of VH domains, which methods can also be applied in an analogous manner to VHH domains from Camelids and to Nanobodies, are the method described by Chothia et al. (Nature 342, 877-883 (1989)), the so-called "AbM definition" and the so-called "contact definition". However, in the present description, aspects and figures, the numbering according to Kabat as applied to VHH domains by Riechmann and Muyldermans will be followed, unless indicated otherwise.

It should also be noted that the Figures, any Sequence Listing and the Experimental Part/Examples are only given to further illustrate the invention and should not be interpreted or construed as limiting the scope of the invention and/or of the appended claims in any way, unless explicitly indicated otherwise herein.

It should further be noted that the present invention is not specifically limited to any causation, explanation, hypothesis or mechanism of/for the protein interference (and/or signals arising in immunoassays) that is observed in, and that may be reduced according to, the present invention. However, it is assumed that the blood or serum (or other biological fluids, such as those mentioned herein) of certain individuals or groups of individuals may contain certain (pre-existing) proteins that under certain circumstances may (aspecifically) bind to ISV's leading to a interfering signal in certain assays that are used to analyze blood or serum samples obtained from such individuals. This is *inter alia* based on the observation made in establishing the present invention that the aspecific protein interference that is addressed by the present invention not only occurs when assaying samples that have been obtained from subjects to which an ISV has previously been administered, but also when assaying sample that have been obtained from subjects that have not previously received an ISV.

In particular, based on the observations that have been made in establishing the present invention, and although the invention is not limited thereto, it is thought that such (pre-existing) proteins may in particular (be able to) bind to the C-terminal end of such ISV's (which, in full sized conventional 4-chain monoclonal antibody as well as in the "heavy-chain only" antibodies that are found in *Camelidae*, are linked to the rest of the antibody - i.e. to the CH1 region in conventional monoclonals and to the hinge region in Camelidae heavy chain

antibodies, respectively - and thus in such full-sized antibodies may be shielded from such protein interference).

This is confirmed by the findings made by the present inventors in establishing the present invention (which findings are further described herein) that certain (simple) modifications of ISV's at their C-terminal end may substantially reduce or essentially prevent such protein interference. Accordingly, methods for modifying ISV's in this manner as well as ISV's that have been modified in this manner form further aspects of the invention, as further described herein.

The present invention can in particular be used to reduce or avoid protein interference and/or signals due to aspecific binding in immunoassays that are performed on biological samples (such as blood or serum samples) obtained from a subject to whom a (biological) drug has been administered (again, such samples are also referred to herein as the "test sample" or "assay sample"). Some examples of this are immunoassays that are used for characterization of drug disposition and of the formation of antibodies upon administration of a biological drug to a subject, such as those referred to in the "*Guideline on the Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins*" (document CHMP/EWP/89249/2004 dated January 27, 2007) issued by the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMEA). As stated on pages 4 and 5 of this document:

> "*Several possible weaknesses have been identified and may result in erroneous characterisation of drug disposition and of the formation of antibodies. The following issues should be considered […]*:
>
> *Immunoassay*
>
> *Drug assay:*
>
> *[…]*
>
> *(iii) Interference by endogenous substances.*
>
> *(iv) Interference by plasma components or anti-drug antibodies binding to the analyte and inhibiting the complementary binding to capture antibody.*"

The invention can in particular be used in order to predict, reduce or avoid this type of interference in immunoassays that are used in analyzing test samples/assay samples of biological fluids taken from subjects to whom ISV's (and in particular Nanobodies; or an ISV-based biological or Nanobody-based biological, as further defined herein) have been administered.

The invention can in particular be used in order to predict, reduce or avoid this type (aspecific) protein interference in immunoassays that are used for characterization of drug disposition and/or for determining the formation of any ADA's (anti-drug) antibodies. In this respect, should be noted that generally in this specification and in the attached claims, when wording like "predicting, reduce or avoiding protein interference" is used, this does not only include predicting, reduce or avoiding such protein interference *per se*, but also generally predicting, reduce or avoiding the occurrence of aspecific signals in immunoassays (such as those in which (aspecific) signals associated with protein interference may occur, for example in ADA assays), and in particular predicting, reduce or avoiding, in such immunoassays, the occurrence of aspecific signals that, when they are observed in such an assay, are usually attributed to, associated with and/or taken as a sign of (aspecific) protein interference. In this respect, it should generally be noted that, as mentioned herein, the present invention is not specifically limited to any causation, explanation, hypothesis or mechanism.

In one specific but non-limiting aspect, the invention can be used to predict, avoid or reduce such protein interference in "anti-drug antibody" or "ADA" assays that are performed on samples (*i.e.,* "test samples") of biological fluids taken from subjects to whom ISV's (and in particular Nanobodies; or an ISV-based biological or Nanobody-based biological, as further defined herein) have been administered.

In another specific but non-limiting aspect, the invention can be used to predict, avoid or reduce such protein interference (and/or aspecific signals usually associated with the same) in "anti-drug antibody" or "ADA" assays that are used to detect, measure and/or characterize the presence of (any) anti-drug antibodies against one or more ISV's (and in particular against Nanobodies; or an ISV-based biological or Nanobody-based biological, as further defined herein). In particular, the invention can be used to predict, avoid or reduce such protein interference in such "anti-drug antibody" or "ADA" assays that are performed on samples (*i.e.,* "test samples") of biological fluids, and more in particular on samples of biological fluids of that have been obtained from a subject to whom one or more such ISV's

or Nanobodies (or an ISV-based biological or Nanobody-based biological, as further defined herein) have been administered. For example, the invention can be used to predict, avoid or reduce such protein interference in such "anti-drug antibody" or "ADA" assays that are used to detect, measure and/or characterize the presence of (any) anti-drug antibodies against the ISV or Nanobody (or an ISV-based biological or Nanobody-based biological, as further defined herein) that has been administered to the subject from which the sample has been obtained (either in the context of a clinical trial and/or in the context of therapy).

Thus, in one specific, but non-limiting aspect, the invention can be used to predict, avoid or reduce such protein interference (and/or aspecific signals usually associated with the same) in biological samples (*i.e.,* "test samples") obtained from a subject to whom one or more such ISV's or Nanobodies (or an ISV-based biological or Nanobody-based biological, as further defined herein) have been administered, wherein said samples as suitable for and/or intended for use in an immunological assay, such as an ADA assay. As mentioned, such a biological sample may be blood (including whole blood, serum or plasma), ocular fluid, bronchoalveolar fluid/BALF, cerebrospinal fluid or any other suitable biological fluid or sample that is suitable for use in an immunoassay, and in particular an ADA assay.

In one specific, but non-limiting aspect, such a test sample may have been obtained from a subject that has been subjected to multiple administrations (for example at least 1 to 3 separate administrations over a period of at least 10 days, such as at least one month or longer) and/or to chronic treatment (i.e. treatment during at least 10 days such as at least one month) with an ISV, Nanobody, an ISV-based biological (as further defined herein) or Nanobody-based biological (as further defined herein). Such an ISV, Nanobody, ISV-based biological or Nanobody-based biological may for example have been administered to said subject in the context of therapy or in the context of a clinical trial.

In one specific, but non-limiting aspect, such a test sample may have been obtained from a subject to which a ISV, Nanobody, ISV-based biological or Nanobody-based biological has been administered that has (and/or has been provided with) an increased half-life (as defined herein, and compared to a monovalent ISV), for example a half-life of at least 1 day, preferably at least 3 days, more preferably at least 7 days, such as at least 10 days in the subject to which the same is/has been administered.

For example and without limitation, such an ISV, Nanobody, ISV-based biological or Nanobody-based biological may have been provided with an increased half-life by

functionalization and/or by including in the construct a moiety or binding unit that increases the half-life of the construct. Examples of such functionalization, moieties or binding units will be clear to the skilled person and may for example be as described herein, and for example may include pegylation, fusion to serum albumin, or fusion to a peptide or binding unit that can bind to a serum protein such as serum albumin. Such a serum-albumin binding peptide or binding domain may be any suitable serum-albumin binding peptide or binding domain capable of increasing the half-life of the construct (compared to the same construct without the serum-albumin binding peptide or binding domain), and may in particular be serum albumin binding peptides as described in WO 2008/068280 by applicant (and in particular WO 2009/127691 and the non-prepublished US application 61/301,819, both by applicant), or a serum–albumin binding ISV (such as a serum-albumin binding Nanobody; for example Alb-1 or a humanized version of Alb-1 such as Alb-8, for which reference is for example made to WO 06/122787).

Thus, in one specific but non-limiting aspect, such a biological sample may have been obtained from a subject to which an ISV, Nanobody, ISV-based biological or Nanobody-based biological has been administered that comprises a (human) serum albumin-binding binding peptide or binding domain.

As already mentioned above, in one non-limiting aspect, the invention generally relates to a method that can be used to predict whether a given ISV or Nanobody (or ISV-based or Nanobody-based drug) will give rise to (or has high or increased tendency to give rise to) protein interference (as further described herein) in an immunoassay (i.e. after said ISV has been administered to a subject, a sample of a biological fluid has been obtained from said subject, and said biological fluid is subjected to an immunoassay as further described herein), said method comprising performing an immunoassay that at least comprises the steps of:

(i) contacting said ISV or Nanobody (or ISV-based or Nanobody-based drug) with an antibody that has been obtained from a human subject and that has been selected, generated and/or isolated based on its ability to recognize and/or bind to the C-terminal end of an ISV or Nanobody (the "*analytical antibody*"); and

(ii) determining whether said ISV or Nanobody (or ISV-based or Nanobody-based drug) is bound by said antibody in said immunoassay.

Again, as mentioned herein, an ISV as described herein may in particular either be a Nanobody or an(other) ISV (i.e. other than a Nanobody) that is a VH domain or that comprises a VH domain; and is preferably a Nanobody.

Also, any protein or polypeptide that comprises an ISV (such as an ISV-based drug) preferably has said (or at least one) such ISV at its C-terminal end. Again, said ISV may in particular either be a Nanobody or an(other) ISV (i.e. other than a Nanobody) that is a VH domain or that comprises a VH domain; and is preferably a Nanobody.

In an alternative embodiment, which is also further described herein, instead of the aforementioned antibody obtained from a human subject, the monoclonal antibody referred to herein as "21-4-3" (or "21-4" for short, see SEQ ID NO's 35 and 36 for the VH and VL sequences) may be used. 21-4 was generated using hybridoma technology starting from a mouse immunized with the Nanobody construct of SEQ ID NO:98 in WO 2006/122825, as further described in Example 7, and a hybridoma cell line (called "ABH0015") expressing 21-4 has been deposited on June 4, 2012 with the BCCM, Ghent, Belgium, under accession number LMBP-9680-CB. Monoclonal 21-4 has been shown to recognize the C-terminus the Nanobody construct of SEQ ID NO:98 in WO 2006/122825, which C-terminal end consists of a Nanobody (humanized V_{HH}) raised against Von Willebrand Factor (vWF). 21-4 was originally raised as analytical reagent for use in detecting the protein Nanobodies (n particular, the Nanobody construct of SEQ ID NO:98 in WO 2006/122825) in (serum) samples; surprisingly, it has now been found that 21-4 can also be used in order to predict whether an ISV has a tendency to undergo aspecific protein interference (more so than some other, comparable (mouse) monoclonals raised against the Nanobody construct of SEQ ID NO:98 in WO 2006/122825 or against other Nanobodies).

In particular, it has been found that if measuring the binding of 21-4 to an ISV (or to protein or polypeptide containing an ISV at its C-terminal end, or similar protein or polypeptide as mentioned herein) gives an RU value of less than 500 (after adjusting the measured RU value for the molecular weight to the protein, according to the formula [RU measured]/[MW of the protein] x 10^6) when determined according to the protocol set out in Example 9, that said ISV or protein will likely not have a tendency to undergo protein interference (within the confidence provided by the data set out in the Examples below). For the purposes of the above formula, MW may be calculated as the sum of all the MW's of all the amino acid residues present in the ISV.

Accordingly, any ISV, protein or polypeptide described herein preferably has such an RU value for binding by 21-4 of less than 500 (determined according to the protocol set out in Example 9, and after adjusting the measured RU value for the molecular weight of the ISV or protein used according to the formula set out above).

Thus, this aspect of the invention generally relates to a method that can be used to predict whether a given ISV or Nanobody (or ISV-based or Nanobody-based drug) will give rise to (or has high or increased tendency to give rise to) protein interference (as further described herein) in an immunoassay (i.e. after said ISV has been administered to a subject, a sample of a biological fluid has been obtained from said subject, and said biological fluid is subjected to an immunoassay as further described herein), said method comprising performing an immunoassay that at least comprises the steps of:

(i) contacting said ISV or Nanobody (or ISV-based or Nanobody-based drug) with the monoclonal antibody 21-4 (i.e. used as the "*analytical antibody*"); and

(ii) determining whether said ISV or Nanobody (or ISV-based or Nanobody-based drug) is bound by the monoclonal antibody 21-4 in said immunoassay.

Said method may in particular be performed using BiaCore or a similar technique, and more in particular using the protocol set out in Example 9. As mentioned herein, when the binding of the ISV or ISV-based drug in this protocol shows an RU value of less than 500 (after adjusting the measured RU value for the molecular weight to the protein, according to the formula [RU measured]/[MW of the protein] x 10^6), said ISV or ISV-based protein will likely not be bound by any interference factor(s) present in the blood or serum of a human being and/or will likely not have a tendency to undergo aspecific protein interference in an ADA assay (i.e. within the degrees of confidence set out in the experimental part below).

Again, as mentioned herein, an ISV as described herein may in particular either be a Nanobody or an(other) ISV (i.e. other than a Nanobody) that is a VH domain or that comprises a VH domain; and is preferably a Nanobody.

Also, any protein or polypeptide that comprises an ISV (such as an ISV-based drug) preferably has said (or at least one) such ISV at its C-terminal end. Again, said ISV may in particular either be a Nanobody or an(other) ISV (i.e. other than a Nanobody) that is a VH domain or that comprises a VH domain; and is preferably a Nanobody.

As also mentioned herein, the above method using 21-4 can also be used to determine whether an ISV or protein or polypeptide that comprises a ISV is bound by (or has a tendency to be bound by) interference factor(s) that are present in the blood or serum of a human being.

Also, as mentioned herein, it is envisaged that said method using 21-4 can also be used to predict whether any protein or polypeptide (such as an antibody fragment or ScFv) that has a VH domain at its C-terminal end will bound by (or has a tendency to be bound by) interference factor(s) that are present in the blood or serum of a human being and/or has a tendency to undergo protein interference in an ADA assay.

In addition to 21-4, it is envisaged that an antibody or antibody fragment (such as a suitable Fab fragment) that contains the heavy chain and light chain variable domains of 21-4 (see SEQ ID NO's: 35 and 36, respectively) or even only the CDR sequences of 21-4 (suitably grafted into other suitable VH and VK frameworks) may also be used in the methods described herein.

As further described herein, the invention can in particular be used to predict whether a given ISV or Nanobody (or ISV-based or Nanobody-based drug) will give rise to protein interference (as further described herein) in an immunoassay that is an ADA assay. Said ADA assay may for example be an ADA assay for detecting or measuring ADA's against ISV's generally, and may in particular be an ADA assay for detecting or measuring ADA's against the ISV used in steps (i) and (ii) above.

In one particular preferred but non-limiting aspect, the invention can be used to predict whether a given ISV or Nanobody (or ISV-based or Nanobody-based drug) will give rise to protein interference (as further described herein) in an immunoassay (and in particular, in an ADA assay) that involves the use of such an ISV. Again, said ADA assay may for example be an ADA assay for detecting or measuring ADA's against ISV's generally, and may in particular be an ADA assay for detecting or measuring ADA's against the ISV used in steps (i) and (ii) above.

In an even more particular but non-limiting aspect, the invention can be used to predict whether a given ISV or Nanobody (or ISV-based or Nanobody-based drug) will give rise to protein interference (as further described herein) in an immunoassay (and in particular, in an ADA assay) that involves the use of such an ISV. For example, such an immunoassay may be an ADA assay (i.e. involving the ISV) that is performed to determine or measure whether any ADA's against said ISV are present in the sample that is tested, wherein said

sample is a sample of biological fluid (as described herein) that is obtained from a subject to which said ISV has been administered (as further described herein). For example, as further mentioned herein, said sample (*i.e.,* the "test sample") may be a sample of (including whole blood, serum or plasma), ocular fluid, bronchoalveolar fluid/BALF, cerebrospinal fluid or any other suitable biological fluid, and may in particular be a biological sample that is suitable for and/or intended for use in an immunological assay, such as an ADA assay.

As further described herein, in all these aspects (and the further aspects of the invention described herein), the invention can also be used to select ISV's that are not or less prone to such protein interference in such immunoassays or ADA assays; as an assay or test that can be used to test whether certain modification(s) to an ISV will (fully or partially) reduce its tendency to give rise to such interference in such immunoassays or ADA assays; and/or as an assay or test that can be used to guide modification or improvement of an ISV so as to reduce its tendency to give rise to such protein interference in such immunoassays or ADA assays.

As mentioned, step (i) of the method of the invention comprises contacting said ISV or Nanobody (or ISV-based or Nanobody-based drug) with an antibody that has been obtained from a human subject and that has been selected/isolated based on its ability to recognize and/or bind to the C-terminal end of an ISV or Nanobody (as further described herein). In said step (i) of the method described herein, "*said ISV or Nanobody (or ISV-based or Nanobody-based drug)*" is used as the antigen in the immunoassay (i.e. as the substance to be detected). Also, in said step (i), the "*antibody that has been obtained from a human subject and that has been selected/isolated based on its ability to recognize and/or bind to the C-terminal end of an ISV or Nanobody*" is used as the analytical reagent (i.e. in the same way as other antibodies are used in immunoassays to detect the presence of an antigen to which they are directed).

As already mentioned, and in order to better understand the invention described herein, it should be noted that, in step (i), the ISV will usually be used as the "antigen" (*i.e.,* as the compound to be detected), and the "analytical antibody" will be used as the analytical agent (*i.e.,* as a means to detect whether a given ISV binds or not, respectively; and thus has a high or increased risk of giving rise to protein interference or not, respectively). For example, when step (i) is performed in an ELISA format, the "antibody/analytical agent" will usually

be bound to the carrier (*i.e.,* to the ELISA plate) and the ISV will be (present in) the sample to be tested.

By contrast, it should be noted that in ADA assays for detecting or measuring ADA's against an ISV, the ISV is used as the "analytical agent" (*i.e.,* as the compound used to detect whether any ADA's are present), and the ADA's are the "antigen" (*i.e.,* the compound to be detected). Thus, in these assays, the ISV will usually/often be bound to the carrier (such as the ELISA plate), whereas the ADA's (if any) will be present in the sample that is subjected to the assay.

However, as already mentioned, it should generally be noted that the invention is not limited to assays in which the "analytical antibody" is bound to the carrier. For example, in an alternative way of performing an assay according to the invention (as shown in Example 5), the analytical antibody is instead used as a bridging agent and thus will be in solution rather than bound to the plate (although it is indirectly bound to the plate via the ISV that is coated on the plate). However, also in the specific (bridging) assay described in Example 5 (which is a competitive assay) the analytical antibody is still used as the analytical agent (*i.e.,* to determine whether the ISV of interest binds or not, respectively; and thus has a high or increased risk of giving rise to protein interference or not, respectively). It is also envisaged that, based on the further disclosure herein, the skilled person will be able to design other assay formats in which the analytical antibody can be used as an analytical agent in order to determine whether a given ISV can bind or not, respectively; and thus has a high or increased risk of giving rise to protein interference or not).

The "analytical antibody" used in step (i) may be a polyclonal or monoclonal antibody.

When the analytical antibody is a polyclonal antibody, it may for example be a polyclonal antibody (preparation) that has been obtained/purified/isolated from a biological sample obtained of a human subject (such as blood, plasma, B-cells or another suitable biological sample or fluid from which polyclonal antibodies can be suitably isolated). This may for example be a suitable biological sample that has been obtained of a human subject to which at least one ISV (such as the ISV used in step (i), but this is not required or essential) has been administered, but may also be (and preferably is) a suitable biological sample from a human subject which has never received or been treated with an ISV. What is more important is that the polyclonal antibody has been obtained from said biological sample by a method

that involves at least one affinity step using an affinity matrix or column that carries an ISV as the affinity moiety (and one or more further steps for obtaining/purifying/isolating polyclonal antibodies known per se). For example, the polyclonal antibody may have been obtained from such a biological sample by means of affinity chromatography using an affinity column that carries an ISV, as for example described in Example 2. This may for example be performed using well known techniques for immunoaffinity chromatography for isolating antibodies from a biological sample, using an affinity matrix that carries an ISV as the antigen. Such techniques are generally known in the art and suitable examples thereof will be clear to the skilled person based on the disclosure herein.

Such a polyclonal antibody (preparation) may in particular be an IgG (or IgG fraction).

For example, it may be a polyclonal antibody that has been obtained means of a method that involves (immuno)affinity chromatography, performed on a sample of biological fluid obtained from a human subject, using as the antigen bound to the affinity matrix an ISV (and in particular a Nanobody, such as a VHH, humanized and/or sequence-optimized VHH or a camelized VH, such as a camelized human VH) that does not contain a C-terminal tag (*i.e.,* of which the C-terminus ends with the amino acid sequence VTVSS (SEQ ID NO:33)). In particular, the ISV used as the antigen bound to the affinity matrix may be a humanized or sequence-optimized VHH (or alternatively a corresponding camelized human VH) of which the C-terminus ends with the amino acid sequence VTVSS (SEQ ID NO:33). In one specific, but non-limiting aspect, the ISV used as the antigen bound to the affinity matrix may be a humanized or sequence-optimized VHH that, as a result of such humanization or sequence-optimization, comprises a proline (P) residue at position 14 where the corresponding "naïve" VHH comprises an alanine (A) at position 14 (in other words, the ISV used as the antigen is a humanized version of a VHH that naturally comprises an alanine at position 14, which alanine residue, as a result of the humanization and/or sequence optimization, has been replaced with a proline (P) residue). The ISV used as the antigen may also comprise one or more other amino acid substitutions as a result of such humanization or sequence optimization, for example generally described in WO 08/020079 or WO 09/138519.

Some specific examples of ISVs that can be used as the antigen to generate/isolate the "analytical antibody" used in the invention are given in SEQ ID NO's: 1 and 2.

Again, the method used to obtain the polyclonal antibody may, in addition to the (immune)affinity steps, also comprise one or more further steps for isolating/purifying a polyclonal antibody from the biological sample (performed either before or after the affinity steps). Again, such steps and techniques for performing them will be clear to the skilled person.

Thus, in one aspect, the invention comprises a method as further described herein that comprises steps (i) and (ii) described herein, in which the "analytical antibody" (*i.e.,* the antibody that has been obtained from a human subject and that has been selected/isolated based on its ability to recognize and/or bind to the C-terminal end of an ISV or Nanobody) has been obtained from a biological sample obtained from a human subject (wherein said biological sample is a sample that is suitable for use in a method for generating/isolating an antibody from said sample) using a method that comprises at least one affinity step (such as a step of affinity chromatography, such as immunoaffinity chromatography) in which an ISV (and preferably a Nanobody) is used as an antigen, and preferably an ISV is used as an antigen that comprises the amino acid sequence VTVSS (SEQ ID NO:33) as the C-terminal sequence, and more preferably a humanized and/or sequence optimized Nanobody is used as the antigen that comprises the amino acid sequence VTVSS (SEQ ID NO:33) as the C-terminal sequence, and even more preferably a humanized and/or sequence optimized Nanobody is used as the antigen that comprises the amino acid sequence VTVSS (SEQ ID NO:33) as the C-terminal sequence and that comprises a proline residue at position 14, such as a Nanobody that comprises the amino acid sequence VTVSS (SEQ ID NO:33) as the C-terminal sequence and that comprises a proline residue at position 14 that has been introduced into the Nanobody as a result of said humanization and/or sequence-optimization (for example, to replace an alanine residue that naturally occurs at said position in the VHH that has been humanized and/or sequence optimized).

The above ISV's can also be used in methods to isolate monoclonal antibodies (again starting from a suitable biological sample obtained from a human being) that are suitable for use in the invention as the "analytical antibody".

For example, such a monoclonal antibody may be obtained starting from blood, B-cells or another suitable sample or material for isolating antibodies, may be selected based on its ability to recognize and/or bind to (the C-terminal end of) an ISV or Nanobody (in which, again, the ISV(s) used as the antigen during screening and/or selection is preferably as

described in the preceding paragraphs, including the preferences stated for such ISV/antigen). Such screening and selection may be performed in any suitable manner, for example by using B-cell selection and/or expansion techniques essentially the same or suitably similar to the B-cell selection techniques described in EP 0 488 470, WO 92/02551, EP 1 633 787, WO 01/55216, WO 02/26829, WO 04/051268, WO 04/102198 or WO 04/106377 or techniques similar to the Nanoclone technique described in WO 06/079372 (but using human B-cells rather than camelid B-cells).

Once one or more B-cells have been identified/isolated that express a suitable antibody, said antibody may be isolated, expressed and/or produced in any suitable manner. For example, said B-cell(s) may be immortalized as hybridomas producing the desired antibody/antibodies (using techniques well known per se for generating hybridomas starting from selected B-cells), and said antibody/antibodies may then be isolated from (the culture supernatant of) said hybridoma(s), again using suitable techniques well established in the art and described in various handbooks and manuals, and also described and/or referred to in the patent publications mentioned in the preceding paragraph.

Alternatively, said B-cell(s) may be expanded using B-cell expansion techniques known per se, and the antibody/antibodies may be isolated from (the culture supernatant of) said expanded B-cell(s). Again, this may be performed using suitable techniques well established in the art and described in various handbooks and manuals, and also described and/or referred to in the patent publications mentioned in the preceding paragraphs.

In yet another alternative, DNA encoding the antibody/antibodies of interest may be obtained (*e.g.,* by amplification) from said B-cell(s) or other suitable cells, either directly (for example using suitable single-cell PCR cloning techniques) or after suitable expansion of the desired B-cell(s). Said DNA may then be suitably expressed in a suitable host cell or host organism to provide the desired antibody/antibodies. Again, this may be performed using suitable techniques well established in the art and described in various handbooks and manuals, and also described and/or referred to in the patent publications mentioned in the preceding paragraphs.

It is also possible to generate monoclonal antibodies that are suitable for use as the "analytical antibody" by a method that involves repertoire cloning (starting from a suitable sample obtained from a human subject) and screening the cloned repertoire for antibodies that bind to the ISV used as antigen (in which, again, the ISV(s) used as the antigen during

screening and/or selection is preferably as described in the preceding paragraphs, including the preferences stated for such ISV/antigen). Methods for repertoire cloning and various techniques for displaying cloned repertoires for selection and screening (such as phage display, ribosome display and yeast display) will be clear to the skilled person, and are for example described in EP 0 589877, EP 0 774 511, WO 90/14430 and EP 0368 684) as well as various handbooks on the subject.

Generally, the biological sample that is used as a starting point for obtaining the (polyclonal or monoclonal) analytical antibody may be any suitable sample (i.e. suitable as a starting material for obtaining a polyclonal or monoclonal antibody, respectively) obtained from any suitable human subject. In one specific but non-limiting aspect, such a sample may for example have been obtained from a woman, and in particular a post-menopausal woman. Thus, in one specific but non-limiting aspect, the analytical antibody used in steps (i) and (ii) above has been obtained starting from a biological sample that has been obtained/derived from a post-menopausal woman (or has been derived from an antibody that has been obtained/derived from a post-menopausal woman).

Also, the biological sample that is used as a starting point for obtaining the (polyclonal or monoclonal) analytical antibody may be obtained from a subject to whom an ISV has previously been administered (for example, as part of a clinical trial or therapeutically), but is preferably obtained from a subject to whom no ISV has previously been administered.

However, it should be noted that the invention is not particularly limited to the source of the analytical antibody/antibodies used, and it has proven possible in some cases, using the techniques described herein, to obtain (generate, isolate) other suitable analytical antibodies from other sources, including commercially available human blood or plasma (and even blood, plasma or B-cells from other species of mammals or primates, such as from baboon or cynomolgus monkey).

As mentioned above, the (polyclonal or monoclonal) analytical antibody used in steps (i) and (ii) should be such that it is capable of recognizing or binding to the C-terminal end of an ISV or Nanobody, and is most preferably selected and/or isolated based on this ability to bind to the C-terminal end of an ISV or Nanobody.

As can be seen from Figure 2, when the ISV is based on or derived from a VH or VHH domain, the C-terminal end of an ISV comprises the amino acid sequence VTVSS

(SEQ ID NO:33), and accordingly the analytical antibody should be capable of recognizing any ISV that has the amino acid sequence VTVSS (SEQ ID NO:33) at its C-terminal end. As can be further seen from Figure 2, (at least some of the amino acid residues in) the sequence VTVSS (SEQ ID NO:33) is part of a putative epitope on the ISV that also includes, among other residues, the amino acid residue at position 14 (and the amino acid residues next/close to the same in the amino acid sequence, such as positions 11, 13 and 15) and may also comprise the amino acid residue at position 83 (and the amino acid residues next/close to the same in the amino acid sequence, such as positions 82, 82a, 82b and 84) and/or the amino acid residue at position 108 (and the amino acid residues next/close to the same in the amino acid sequence, such as positions 107. Position 109 is the first V of the C-terminal VTVSS (SEQ ID NO:33) sequence and it has been shown that for example position 110 may have an influence on protein interference as well). This is also collectively referred to herein as the "*C-terminal region*", it being understood that this C-terminal region at least comprises the C-terminal sequence VTVSS (SEQ ID NO:33) and the amino acid residue at position 14, and may also comprise the amino acid residues at positions 83 and 108, and possibly also the amino acid residues at positions 13, 15, 82b, 83, 84 and 107.

As already mentioned, and again without being limited to any hypothesis or explanation, in a full-sized 4-chain monoclonal antibody, or in a full-sized heavy chain only antibody such as those present in *Camelidae*, the C-terminal end of a VH or VHH domain is linked to the rest of the antibody - i.e. to the CH1 region in conventional monoclonals or to the hinge region in Camelidae heavy chain antibodies, respectively - and thus in such full-sized antibodies may be shielded from such protein interference) and/or covered by the VH/VL interaction (in conventional 4-chain antibodies) so that this "C-terminal region" and is therefore usually not solvent-exposed and/or accessible as an interaction site for proteins that are present in the blood, serum or body of a person to which such an ISV is administered. However, if an ISV or Nanobody is used per se (i.e. without being linked to any other part of an antibody), or if an ISV-based drug or Nanobody-based drug is used that carries an ISV or Nanobody at its C-terminal end, this C-terminal epitope is available for (aspecific) interaction with other proteins, and again without being limited to any hypothesis or explanation, it is assumed that this C-terminal region may now be accessible to undergo an (aspecific) protein interaction with one or more proteins that are pre-existing in the "test sample" (for example,

one or more IgG's) to be tested and that this may cause protein interference and/or aspecific signals in the immunoassays (and in particular in ADA assays).

As mentioned, the methods described herein can be used to predict, reduce or avoid such protein interaction, and can also be used as a tool to guide modification to the ISV, Nanobody, ISV-based drug or Nanobody-based drug so as to provide the same with a (partially or preferably essentially fully) reduced tendency to give rise to such protein interference.

As will be clear from the preceding paragraph, and again without being limited to any hypothesis or explanation, it is in particular expected (and part of the teaching of the present invention) that (certain) modifications to the "C-terminal region" will alter (and preferably reduce) the tendency of an ISV to undergo such aspecific protein interaction, and this is also what is observed experimentally (see for example the experimental results presented in Examples 1C and 3 below).

Based on this, and again without being limited to any hypothesis or explanation, the present invention also teaches certain modifications that can be introduced for this purpose in the C-terminal region of an ISV, Nanobody, ISV-based drug or Nanobody-based drug (of which the (potential) effectiveness can be tested using the methods described herein). Also, based on the teaching herein, it is envisaged that the skilled person will be able to choose, design or propose other (candidate) modifications to the C-terminal region that could be introduced for this purpose (and of which the (potential) effectiveness can again be tested using the methods described herein).

Returning to the analytical antibody used in the invention, this is preferably a (polyclonal or monoclonal) antibody that recognizes the C-terminal region (as defined above) of an ISV, and in particular but without limitation the C-terminal region of a Nanobody.

For example, in one specific but non-limiting aspect, the "analytical antibody" may be a polyclonal or monoclonal that recognizes (and/or is capable of binding to, and in particular of specific binding to) the C-terminal region of an ISV or Nanobody of which the C-terminal end of the sequence ends with VTVSS (SEQ ID NO:33), but does not recognize (and/or is not capable of specific binding to) the C-terminal region of an ISV or Nanobody (which may be a different ISV but is preferably the same ISV) when there are one or more further amino acid residues (such as 1 to 5 amino acid residues, or alternatively a small peptide sequence or even another polypeptide or protein) linked to the C-terminal VTVSS (SEQ ID NO:33).

In another, more specific but still non-limiting aspect, the "analytical antibody" may be a polyclonal or monoclonal that recognizes (and/or is capable of binding to, and in particular of specific binding to) the C-terminal region of an ISV or Nanobody of which the C-terminal end of the sequence ends with VTVSS (SEQ ID NO:33) and in which position 14 is an amino acid that does not naturally occur at position 14 and/or has been modified compared to the amino acid that naturally occurs at position 14 (for example as a result of humanization, camelization and/or sequence optimization), but that does not recognize (and/or is not capable of specific binding to) the C-terminal region of an ISV or Nanobody (which may be a different ISV but is preferably the same ISV) in which there are one or more further amino acid residues (such as 1 to 5 amino acid residues, or alternatively a small peptide sequence or even another polypeptide or protein) linked to the C-terminal VTVSS (SEQ ID NO:33); and/or in which position 14 is an amino acid that naturally occurs at position 14 (for example alanine or, when the ISV naturally contains a proline at position 14, proline).

For example, the "analytical antibody" may also be a polyclonal or monoclonal that recognizes (and/or is capable of binding to, and in particular of specific binding to) the C-terminal region of an ISV or Nanobody of which the C-terminal end of the sequence ends with VTVSS (SEQ ID NO:33) and in which position 14 is proline (and in particular when position 14 has been modified to proline, for example as a result of humanization, camelization and/or sequence optimization), but does not recognize the C-terminal region of an ISV or Nanobody (which may be a different ISV but is preferably the same ISV) in which there are one or more further amino acid residues (such as 1 to 5 amino acid residues, or alternatively a small peptide sequence or even another polypeptide or protein) linked to the C-terminal VTVSS (SEQ ID NO:33); and/or in which position 14 is alanine.

The "analytical antibody" may also be a polyclonal or monoclonal that recognizes (and/or is capable of binding to, and in particular of specific binding to) the C-terminal region of an ISV or Nanobody of which the C-terminal end of the sequence ends with VTVSS (SEQ ID NO:33) and in which position 14 is proline (in particular where a proline residue naturally occurs at said position in said ISV), but does not recognize the C-terminal region of an ISV or Nanobody (which may be a different ISV but is preferably the same ISV) in which there are one or more further amino acid residues (such as 1 to 5 amino acid residues, or alternatively a small peptide sequence or even another polypeptide or protein) linked to the C-terminal

VTVSS (SEQ ID NO:33) in which position 14 is still a (naturally occurring or unmodified) proline.

The "analytical antibody" may also for example be a polyclonal or monoclonal that recognizes (the C-terminal region of) the sequence of the ISV called "Nb 3.4" herein (SEQ ID NO: 5) but does not recognize (the C-terminal region of) the sequence of the ISV called "Nb 3.1" herein (SEQ ID NO: 3) and/or (and preferably and) does not recognize the sequence of the ISV called "Nb 3.2" herein (SEQ ID NO: 4).

For the above purpose, whether an "analytical antibody" does (or does not) recognize an ISV or Nanobody (and/or is or is not capable of (specifically) binding to an ISV or Nanobody) can be determined using any suitable binding assay (such as Biacore), but may also be determined using either the BIACORE assay described in example 3 or an ADA assay such as the ADA bridging/competition assay described in Example 5 (See also Figure 1A to 1C and in particular Figure 1B).

Suitable formats/techniques for performing such an assay will be clear to the skilled person based on the disclosure herein, and for example include (without limitation):

- A colorimetric assay such as ELISA with analytical antibody coated directly or indirectly to the plate and detection of bound ISV with monoclonal or polyclonal anti-ISV antibody. Other useful alternative technologies for this setup include but are not limited to electrochemiluminescence (the MSD platform), Fluorescence (DELFIA, GYROS), and other methods that rely on secondary detection of the bound ISV.
- A Surface Plasmon Resonance (such as BIACORE) or other real-time biosensor method (i.e. other than using SPR) with directly or indirectly immobilized analytical antibody and monitoring the binding of subsequently injected/administered ISV. These methods do not need further detection of the bound ISV. A representative method for performing this type is assay is described in Example 3.
- Analyzing the competitive behavior of the ISV in a bridging assay (ADA assay) using the analytical antibody instead of ADA containing biological fluid. For the bridging assay one can make use of different technologies such as ELISA, the MSD platform. Representative methods for performing this type are schematically shown in Figures 1A to 1C and one specific example of this kind of assay is also described in Example 5.
- Any chromatographic method in which the analytical antibody is immobilized on the chromatographic matrix and specific capturing/isolation of ISV from a solution.

Once a suitable analytical antibody has been obtained using one of the methods described herein or in one of the examples (or a method essentially equivalent to the same), said analytical antibody can be used to determine whether a given ISV or Nanobody (or ISV-based or Nanobody-based drug) will give rise to (or has high or increased tendency to give rise to) protein interference (as defined herein), i.e. by performing steps (i) and (ii) described above. As already described herein, this generally involves contacting said ISV, Nanobody, ISV-based drug or Nanobody-based drug with the analytical antibody and determining whether said ISV, Nanobody, ISV-based drug or Nanobody-based drug is recognized by (and/or is bound by, and in particular specifically bound by) said analytical antibody (and in particular whether the C-terminal region of said ISV or Nanobody or of any ISV or Nanobody that forms the C-terminal end of said ISV-based drug or Nanobody-based drug is recognized by said analytical antibody).

This can generally be performed using any suitable technique for determining whether an antigen (in the case, the ISV, Nanobody, ISV-based drug or Nanobody-based drug) is bound by an antibody, and suitable (immune)assay techniques will be clear to the skilled person. Some non-limiting examples are suitable ELISA techniques (including for example sandwich ELISA's); in which, depending on the ELISA format used (as will be clear to the skilled person), either the analytical antibody or the ISV may be coated on the plate and either the analytical antibody or the ISV may be detectably labeled. Other techniques may for example involve the use of a BIAcore instrument (in which again either the analytical antibody or the ISV may be coated on the chip, see for example Example 3). Another alternative may be a competitive bridging assay (as for example exemplified in Example 5), in which the ability is tested of the ISV to compete with another ISV, Nanobody, ISV-based drug or Nanobody-based drug that is known to be bound by the analytical antibody (or visa versa). These and other suitable techniques for determining whether a given ISV, Nanobody, ISV-based drug or Nanobody-based drug is (specifically) bound or recognized by the analytical antibody will be clear to the skilled person based on the disclosure herein.

It will also be clear, based on the disclosure herein, that the present invention (and in particular the analytical antibody used in the present invention) can be used to determine whether or not a given ISV, Nanobody, ISV-based drug or Nanobody-based drug contains an interaction site (such as an interaction site present at or within the C-terminal region, and/or of which the C-terminal region forms part) that is capable of undergoing an (aspecific)

protein interaction with one or more proteins or other components that may be present in a biological sample (*i.e.,* a "test sample") obtained from a subject that is to be subjected to an immunoassay such as an ADA assay (in particular, an ADA assay for determining the presence of any ADA's against the ISV, Nanobody, ISV-based drug or Nanobody-based drug). Thus, when an ISV, Nanobody, ISV-based drug or Nanobody-based drug is recognized by the analytical antibody used in the invention, it is very likely that said ISV, Nanobody, ISV-based drug or Nanobody-based drug contains such an (accessible or exposed) interaction site, and thus will have a tendency to give rise to such protein interference (as defined herein) when it is used in such an immunoassay or ADA assay for testing the test sample. As will be clear to the skilled person, this is something that should preferably be avoided, either by selecting/using another ISV, Nanobody, ISV-based drug or Nanobody-based drug if possible, or by modifying the ISV, Nanobody, ISV-based drug or Nanobody-based such that its tendency to such protein interference will be substantially reduced or essentially removed (again, this can be tested using the method and analytical antibody disclosed herein).

As will also be clear to the skilled person based on the disclosure herein, such a modification may for example comprise making one or more modifications (such as amino acid insertions, additions, deletions or substitutions) to the interaction site on the ISV, Nanobody, ISV-based drug or Nanobody-based drug, such that its ability to undergo an (aspecific) protein interaction with one or more proteins or other components that may be present in a test sample will be reduced or removed. Again, this can be performed by limited trial and error by introducing one or more modifications and then testing whether this ability has been reduced or not, again using the method and analytical antibody disclosed herein. For example, one or more such modifications may be introduced, and then the ability of the modified ISV to bind to the analytical antibody may be compared to that of the original/unmodified ISV. Alternatively, using a competitive bridging format (as for example exemplified in Example 5), or using BIAcore (see for example Example 3), the ability of the modified ISV to (still) compete with the original ISV for binding to the analytical antibody may be determined.

Again, and although the invention is not limited to any hypothesis or explanation, based on the experimental evidence that is set out in the examples below, the inventors have found that this interaction site is likely located at/near the C-terminal region (as defined herein) or that said interaction site forms part of the C-terminal region (or that the C-terminal

region forms part of this interaction site). This is for example based at least in part on the observation that, if an ISV has a tendency to give rise to such protein interference and has VTVSS (SEQ ID NO:33) as the amino acid residues at its C-terminal end, that attaching either a limited number of amino acid residues (such as 1 to 10, for example 1 to 5, such as 1, 2, 3, 4 or 5), or alternatively a tag or another peptide, protein or other moiety to this C-terminal end will usually substantially reduce or essentially remove said tendency. In some cases, it has been found that even adding 1, 2 or 3 amino acid residues to the C-terminal VTVSS (SEQ ID NO:33) (which may be any suitable amino acid(s) or combination of amino acids, which may each be independently chosen from any naturally occurring amino acids such as those listed in Table A-2 on page 64 of WO 09/138519, for example and without limitation from alanine, glycine, valine, leucine or isoleucine) may already substantially reduce or essentially remove said tendency. This is also in part based on the observation that in some cases, where a VHH naturally contains an alanine residue at position 14 (which as mentioned forms part of the C-terminal region; see Figure 2), the naturally occurring VHH often does not have (or has a low) tendency to give rise to such protein interference, whereas a corresponding VHH in which said alanine at position14 has been replaced with a proline residue (for example, for the purposes of humanization or sequence-optimization) can as a result have an increased tendency to give rise to such protein interference (i.e. compared to the VHH with alanine at position 14).

In one aspect, the invention relates to a VHH, a Nanobody (as defined herein, and in particular a humanized VHH or a camelized VH, such as a camelized human VH) or another ISV (or ISV-based drug or Nanobody-based drug with a VHH, Nanobody or other ISV at its C-terminal end) that has been modified (for example, by introducing one or more amino acid substitutions, additions or deletions), and in particular modified in the C-terminal regions (such as by one or more amino acid substitutions or additions in the C-terminal region), such that (i) it has a substantially reduced tendency (such as at least a statistically relevant reduced tendency) to give rise to protein interference (as defined herein); and/or such that (ii) it has, in the method of the invention described herein (such as in the specific assay described in Example 3 or 5), substantially reduced ability to be bound by an analytical antibody as described herein (such as the polyclonal antibody described in Example 2 and used in Examples 3 and 5), in both cases preferably compared to the same VHH, Nanobody or ISV but without the modifications.

Thus, in one aspect, the invention relates to a VHH, a Nanobody (as defined herein, and in particular a humanized VHH or a camelized VH, such as a camelized human VH) or another ISV (or ISV-based drug or Nanobody-based drug with a VHH, Nanobody or other ISV at its C-terminal end) that is a VHH or VH domain (i.e. an ISV that is a VH domain or derived from a VH domain) and/or that has been based on or has been derived from (the amino acid sequence of) a VHH or VH domain, which VHH, Nanobody or ISV comprises the amino acid sequence $VTVSS(X)_n$ (SEQ ID NO:34) at its C-terminal end, in which n is 1 to 10, preferably 1 to 5, such as 1, 2, 3, 4 or 5 (and preferably 1 or 2, such as 1), and in which each X is an (preferably naturally occurring) amino acid residue that is independently chosen (and preferably independently chosen from the group consisting of alanine (A), glycine (G), valine (V), leucine (L) or isoleucine (I); however, as can be seen from the data presented below, other (preferably naturally occurring) amino acid residues or combinations of the aforementioned preferred amino acid residues with other amino acid residues (such as serine, proline, threonine and/or lysine) may also be used). Preferably, said VHH, Nanobody or ISV with the amino acid sequence $VTVSS(X)_n$ (SEQ ID NO:34) at its C-terminal end is such that (i) it has a substantially reduced tendency (such as at least a statistically relevant reduced tendency) to give rise to protein interference (as defined herein); and/or such that (ii) it has, in the method of the invention described herein (such as in the specific assay described in Example 3 or 5), substantially reduced ability to be bound by an analytical antibody as described herein (such as the polyclonal antibody described in Example 2), in both cases preferably compared to the same VHH, Nanobody or ISV but with the amino acid sequence VTVSS (SEQ ID NO:33) at its C-terminal end. Reference is for example made to the assay and data presented in Example 3.

The aforementioned VHH's, Nanobodies or (other) ISVs are preferably such that they have an RU value for binding by 21-4 of less than 500 (determined according to the protocol set out in Example 9, and after adjusting the measured RU value for the molecu It should also be noted that, any time that reference is made in the description herein or in the claims to any C-terminal sequence $VTVSS(X)_n$ (including any of the aspects (a) to (p) above, that according to one specific aspect of the invention, none of the amino acids X is a cysteine residue.

For example, in some preferred aspects, the C-terminal end of the ISV or ISV-containing construct (when this C-terminal end is a VH-derived ISV, VHH or Nanobody) may be:

(a) $VTVSS(X)_n$, in which n = 1 and X = Ala;

(b) $VTVSS(X)_n$, in which n = 2 and each X = Ala;

(c) $VTVSS(X)_n$, in which n = 3 and each X = Ala;

(d) $VTVSS(X)_n$, in which n = 2 and at least one X = Ala (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile);

(e) $VTVSS(X)_n$, in which n = 3 and at least one X = Ala (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile);

(f) $VTVSS(X)_n$, in which n = 3 and at least two X = Ala (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile);

(g) $VTVSS(X)_n$, in which n = 1 and X = Gly;

(h) $VTVSS(X)_n$, in which n = 2 and each X = Gly;

(i) $VTVSS(X)_n$, in which n = 3 and each X = Gly;

(j) $VTVSS(X)_n$, in which n = 2 and at least one X = Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile);

(k) $VTVSS(X)_n$, in which n = 3 and at least one X = Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile);

(l) $VTVSS(X)_n$, in which n = 3 and at least two X = Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile);

(m) $VTVSS(X)_n$, in which n = 2 and each X = Ala or Gly;

(n) $VTVSS(X)_n$, in which n = 3 and each X = Ala or Gly;

(o) $VTVSS(X)_n$, in which n = 3 and at least one X = Ala or Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile); or

(p) $VTVSS(X)_n$, in which n = 3 and at least two X = Ala or Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile);

with aspects (a), (b), (c), (g), (h), (i), (m) and (n) being particularly preferred, with aspects in which n =1 or 2 being preferred and aspects in which n = 1 being particularly preferred.

It should also be noted that, any time that reference is made in the description herein or in the claims to any C-terminal sequence $VTVSS(X)_n$ (including any of the aspects (a) to (p) above, that according to one specific aspect of the invention, none of the amino acids X is a cysteine residue.

Thus, in one preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 1 and X = Ala (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 2 and each X = Ala (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 2 and at least one X = Ala (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile) (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal

end of the sequence $VTVSS(X)_n$, in which n = 3 and at least one X = Ala (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile) (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 3 and at least two X = Ala (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile) (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 3 and each X = Ala (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 1 and X = Gly (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 2 and each X = Gly (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or

is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 3 and each X = Gly (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 2 and at least one X = Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile) (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 3 and at least one X = Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile) (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 3 and at least two X = Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile) (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 2 and each X = Ala or Gly (or a protein or

polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 3 and each X = Ala or Gly (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 3 and at least one X = Ala or Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile) (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end). or

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 3 and at least two X = Ala or Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile) (or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which n = 1, 2 or 3 in which each X = Ala or Gly.

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which:

- n = 1, 2 or 3 in which each X = Ala or Gly; or
- n = 2 or 3 in which all but one X = Ala or Gly (with the remaining amino acid residue X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile)

or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end).

In another preferred aspect, the invention relates to an immunoglobulin single variable domain (ISV), which is either a Nanobody or an(other) ISV that comprises a VH sequence or is derived from a VH sequence (with Nanobodies being preferred) which has a C-terminal end of the sequence $VTVSS(X)_n$, in which:

- n = 1, 2 or 3 in which each X = Ala or Gly; or
- n = 2 or 3 in which at least one X = Ala or Gly (with the remaining amino acid residue X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile);
- n = 2 or 3 in which all but one X = Ala or Gly (with the remaining amino acid residue X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile);

or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end.

In the above aspects, with said (other) "ISV that comprises a VH sequence or is derived from a VH sequence" is meant any ISV that comprises a VH sequence or that is derived from a VH sequence and that is not a Nanobody (i.e. not a VHH, humanized VHH or camelized VH). For example, such (other) ISV may for example be a VH-based (single) domain antibody, VH-based dAb™, or VH-based microbody (see WO 00/29004).

Again, it should be noted that, any time that one of the ISV's referred to herein has a C-terminal sequence $VTVSS(X)_n$ (including without limitation in ISV's referred to in the preceding aspects) that according to one specific aspect of the invention, none of the amino acids X in the sequence $VTVSS(X)_n$ is a cysteine residue.

As further described herein, any such protein or polypeptide may for example be a construct that contains two or more ISV's (such as two or more Nanobodies), optionally linked via one or more suitable linkers. Thus, for example, such a construct may be a bivalent, trivalent, tetravalent or pentavalent construct (such as a bivalent, trivalent,

tetravalent or pentavalent Nanobody construct), and may for example be a bivalent, trivalent, tetravalent or pentavalent construct (such as a bivalent, trivalent, tetravalent or pentavalent Nanobody construct) that is bispecific, trispecific or biparatopic construct (including for example monospecific, bispecific or biparatopic constructs that also can bind to serum albumin (preferred) or another serum protein for half-life extension).

Again, the Nanobodies, ISVs and proteins/polypeptides according to each of the aspects described above are preferably such that they have an RU value for binding by 21-4 of less than 500 (determined according to the protocol set out in Example 9, and after adjusting the measured RU value for the molecular weight of the ISV or protein used according to the formula set out above).

As mentioned herein, it is also envisaged that the invention may also be applied to other proteins or polypeptides (and in particular antibody fragments such as Fab fragments or other proteins or polypeptides based on antibody fragments, such as ScFv's) that have a VH-domain at their C-terminal end. Thus, in another aspect, the invention relates to such a protein or polypeptide (such as an ScFv) that has a VH domain at its C-terminal end with the amino acid sequence $VTVSS(X)_n$ (SEQ ID NO:34) at its C-terminal end, in which n is 1 to 10, preferably 1 to 5, such as 1, 2, 3, 4 or 5, and in which each X is an (preferably naturally occurring) amino acid residue that is independently chosen (and preferably independently chosen from the group consisting of alanine (A), glycine (G), valine (V), leucine (L) or isoleucine (I). Again, according to some specific aspects, said C-terminal end may be according to any of (a) to (p) above, and preferably according to one of (a), (b), (c), (g), (h), (i), (m) or (n), with n being 1, 2 or 3 and preferably 1 or 2.

Again, such proteins or polypeptides are preferably such that they have an RU value for binding by 21-4 of less than 500 (determined according to the protocol set out in Example 9, and after adjusting the measured RU value for the molecular weight of the ISV or protein used according to the formula set out above). Also, again, according to one specific aspect of this aspect of the invention, none of the amino acids X in the C-terminal sequence $VTVSS(X)_n$ is a cysteine residue.

The invention further relates to a pharmaceutical composition that comprises an ISV (and preferably a therapeutic ISV) or a protein or polypeptide comprising at least one ISV (and preferably at least one therapeutic ISV), wherein said ISV, protein or polypeptide is as further described herein (i.e. an ISV, protein or polypeptide according to one or more of the

aspects described herein, and in particular according to one or more of the aspects described on the preceding pages; and more in particular an ISV, protein or polypeptide that has a C-terminal end/sequence that is according to one or more of the aspects described herein), and at least one suitable carrier, diluent or excipient (i.e. suitable for pharmaceutical use), and optionally one or more further active substances. Such compositions, carriers, diluents or excipients can for example be as described in WO 08/020079 for pharmaceutical compositions that comprise a Nanobody or a protein or polypeptide that comprises at least one Nanobody (and as already mentioned, according to the present invention, the ISV is also preferably a Nanobody).

The invention further relates to an ISV or a protein or polypeptide comprising at least one ISV for use in therapy of a disease in a human being (e.g. a patient in need of such therapy), wherein said ISV, protein or polypeptide is as further described herein (i.e. an ISV, protein or polypeptide according to one or more of the aspects described herein, and in particular according to one or more of the aspects described on the preceding pages; and more in particular an ISV, protein or polypeptide that has a C-terminal end/sequence that is according to one or more of the aspects described herein).

The invention further relates to the use of an ISV or a protein or polypeptide comprising at least one ISV in the preparation of a pharmaceutical composition, wherein said ISV, protein or polypeptide is as further described herein (i.e. an ISV, protein or polypeptide according to one or more of the aspects described herein, and in particular according to one or more of the aspects described on the preceding pages; and more in particular an ISV, protein or polypeptide that has a C-terminal end/sequence that is according to one or more of the aspects described herein).

The invention further relates to a method of treatment which comprises administering to a human subject (e.g to a patient in need of such treatment) an ISV or a protein or polypeptide comprising at least one ISV in the preparation of a pharmaceutical composition, wherein said ISV, protein or polypeptide is as further described herein (i.e. an ISV, protein or polypeptide according to one or more of the aspects described herein, and in particular according to one or more of the aspects described on the preceding pages; and more in particular an ISV, protein or polypeptide that has a C-terminal end/sequence that is according to one or more of the aspects described herein); or a pharmaceutical composition (as described above) that comprises at least one such ISV, protein or polypeptide.

With respect to the above, it will be clear that the therapeutic use of the ISV's, proteins and polypeptides described herein are a very important aspect of the invention, as such therapeutic use (or the clinical development of such ISV's, proteins and polypeptides for such therapeutic use) may involve the use of ADA assays to determine whether said ISV, protein or polypeptide is immunogenic (i.e. can give rise to ADA's when administered to a human subject). In this respect, it will also be clear that concerns about possible immunogenicity will in particular have to be addressed when a therapeutic is either used for longer periods of time (for during weeks, months or years), and/or has a half-life (preferably expressed as t1/2-beta) in a human subject of at least 3 days, such as at least one week, and up to 10 days or more.

Thus, according to one specific aspect of the invention, a ISV, protein, polypeptide or pharmaceutical composition as described herein is intended for treatment of a chronic disease in a human being, and/or such ISV, protein, polypeptide as described herein is intended to be present in the circulation of the subject (i.e. at pharmacologically active levels) to which it is administered (i.e. at a therapeutically active dose) for at least a period of one week, preferably at least two weeks, such as at least a months; and/or such ISV, protein, polypeptide as described herein is such that it has a half-life (preferably expressed as t1/2-beta) in a human subject of at least 3 days, such as at least one week, and up to 10 days or more; and/or such an ISV, protein, polypeptide or pharmaceutical composition as described herein is intended to be administered to a human being as two or more doses that are administered over a period of at least 3 days, such as at least one week, for example at least two weeks or at least one month, or even longer (i.e. at least 3 months, at least 6 months or at least one year), or even chronically administered.

The invention further relates to a method for (substantially) reducing or essentially removing the tendency of an ISV, a Nanobody or an ISV-based drug or a Nanobody-based drug to give rise to protein interference, said method comprising at least the steps of:

- optionally determining the tendency of the ISV, Nanobody, ISV-based drug or Nanobody-based drug to give rise to protein interference, using a method that at least comprises steps (i) and (ii) as referred to herein;
- modifying said ISV, Nanobody, ISV-based drug or Nanobody-based drug by introducing one or more one or more amino acid substitutions, additions or deletions in said ISV or Nanobody, or in the C-terminal ISV or Nanobody (if any) of the ISV-based drug or

Nanobody-based drug; and in particular by introducing one or more amino acid substitutions or additions in the C-terminal region of said ISV or Nanobody, or in the C-terminal region of the C-terminal ISV or Nanobody (if any) of the ISV-based drug or Nanobody-based drug, for example by adding to the C-terminal end of the sequence 1 to 10, such as 1 to 5, such as 1, 2, 3, 4 or 5 amino acid residues each independently chosen from any naturally occurring amino acids (such as those listed in Table A-2 on page 64 of WO 09/138519, for example and without limitation from alanine, glycine, valine, leucine or isoleucine);

- determining the tendency of the so modified ISV, Nanobody, ISV-based drug or Nanobody-based drug to give rise to protein interference, using a method that at least comprises steps (i) and (ii) as referred to herein; optionally in a manner that allows the tendency of the so modified ISV, Nanobody, ISV-based drug or Nanobody-based drug to give rise to protein interference to be compared to the tendency of the original ISV, Nanobody, ISV-based drug or Nanobody-based drug to give rise to protein interference (including, without limitation, comparing them in a competition assay for binding to the analytical antibody as described herein). Alternatively, the method described herein that involves the use of 21-4 may be used.

The invention will now be further described by means of the following non-limiting preferred aspects, examples and figures, in which:

- Figure 1A to 1C schematically shows some non-limiting examples of ADA assay formats. Some representative but non-limiting protocols for performing these assays are mentioned in Example 4.
- Figure 2 schematically shows a representative 3D structure of an ISV, such as a Nanobody.
- Figure 3 is a binding curve (obtained using the BIACORE assay described in Example 3) showing the binding of NB's 3.4 to 3.9 (SEQ ID NO's. 5 to 10) to the immobilized polyclonal antibody obtained in Example 2.
- Figure 4 is a binding curve (obtained using the BIACORE assay described in Example 3) showing the binding of NB's 3.4, 3.11, 3.12 and 3.13 (SEQ ID NO's: 5, 12, 13 and 14) to the immobilized polyclonal antibody obtained in Example 2.

- Figure 5 is a binding curve (obtained using the BIACORE assay described in Example 3) showing the binding of NB's 3.4, 3.14 and 3.15 (SEQ ID NO's: 5, 15 and 16) to the immobilized polyclonal antibody obtained in Example 2.
- Figure 6 is a binding curve (obtained using the BIACORE assay described in Example 3) showing the binding of NB's 3.1, 3.2 and 3.4 (SEQ ID NO's: 3, 4 and 5) to the immobilized polyclonal antibody obtained in Example 2.
- Figure 7 is a binding curve (obtained using the BIACORE assay described in Example 3) showing the binding of NB's 4.1 and 4.2 (SEQ ID NO's: 17 and 18) to the immobilized polyclonal antibody obtained in Example 2.
- Figure 8 is a binding curve (obtained using the BIACORE assay described in Example 3) showing the binding of NB's 6.1, 6.2, 6.4 and 6.5 (SEQ ID NO's 19 to 22) to the immobilized polyclonal antibody obtained in Example 2.
- Figure 9 gives is a Table showing the sequences used in Example 8 (SEQ ID NO's: 37 to 89) and setting out the corresponding reference sequence.

The sequences referred to in the present description and claims are listed in Table A below (SEQ ID NO's: 1 to 37) and in Figure 9 (SEQ ID NO's: 38 to 89).

TABLE A

Name	SEQ ID NO:	Sequence
ISV Ex. 1/2-	1	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVSGIKSSGDSTRYAGSVKGRFTISRDNAKNTLYLQMNSL RPEDTAVYYCAKSRVSRTGLYTYDNRGQGTLVTVSSGGGGSG GGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGRTF NNYAMGWFRQAPGKEREFVAAITRSGVRSGVSAIYGDSVKDR FTISRDNAKNTLYLQMNSLRPEDTAVYYCAASAIGSGALRRFE YDYSGQGTLVTVSS
Alt. ISV	2	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYPMGWFRQAPGK GREFVSSITGSGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLR PEDTAVYYCAAYIRPDTYLSRDYRKYDYWGQGTLVTVSSGGG GSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWV RQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYL QMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGS EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYPMGWFRQAPGK GREFVSSITGSGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLR PEDTAVYYCAAYIRPDTYLSRDYRKYDYWGQGTLVTVSS
>Nb3.1	3	EVQLVESGGGLVQAGGSLRLSCAASRSIGRLDRMGWYRHRTG EPRELVATITGGSSINYGDFVKGRFTISIDNAKNTVYLQMNNLK PEDTAVYYCNFNKYVTSRDTWGQGTQVTVSS
>Nb3.2	4	EVQLVESGGGLVQAGGSLRLSCAASRSIGRLDRMGWYRHRTG EPRELVATITGGSSINYGDFVKGRFTISIDNAKNTVYLQMNNLK PEDTAVYYCNFNKYVTSRDTWGQGTQVTVSSAAAEQKLISEED LNGAAHHHHHH
>Nb3.4	5	EVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGWYRHRPGE PRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQMNSLRPE DTAVYYCNFNKYVTSRDTWGQGTLVTVSS

TABLE A (continued)

Name	SEQ ID NO:	Sequence
>Nb3.5	6	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGW YRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQ MNSLRPEDTAVYYCNFNKYVTSRDTWGQGTLVTVSSAA
>Nb3.6	7	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGW YRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQ MNSLRPEDTAVYYCNFNKYVTSRDTWGQGTLVTVSSA
>Nb3.7	8	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGW YRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQ MNSLRPEDTAVYYCNFNKYVTSRDTWGQGTLVTVSSG
>Nb3.8	9	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGW YRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQ MNSLRPEDTAVYYCNFNKYVTSRDTWGQGTLVTVSSGG
>Nb3.9	10	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGW YRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQ MNSLRPEDTAVYYCNFNKYVTSRDTWGQGTLVTVSSGGG
>Nb3.10	11	HHHHHHEVQLVESGGGLVQAGGSLRLSCAASRSIGRLDRMGW YRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQ MNSLRPEDTAVYYCNFNKYVTSRDTWGQGTLVTVSS
>Nb3.11	12	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGW YRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQ MNSLKPEDTAVYYCNFNKYVTSRDTWGQGTLVTVSS
>Nb3.12	13	HHHHHHEVQLVESGGGLVQAGGSLRLSCAASRSIGRLDRMGW YRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQ MNSLRPEDTAVYYCNFNKYVTSRDTWGQGTQVTVSS

TABLE A (continued)

Name	SEQ ID NO:	Sequence
>Nb3.13	14	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGW YRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQ MNSLRPEDTAVYYCNFNKYVTSRDTWGQGTQVTVSS
>Nb3.14	15	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGW YRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQ MNSLRPEDTAVYYCNFNKYVTSRDTWGQGTLVQVSS
>Nb3.15	16	HHHHHHEVQLVESGGGSVQPGGSLRLSCAASRSIGRLDRMGW YRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQ MNSLRPEDTAVYYCNFNKYVTSRDTWGQGTLVTVSS
>Nb4.1	17	EVQLVESGGGLVQPGGSLRLSCAASGSVFKINVMAWYRQAPG KGRELVAGIISGGSTSYADSVKGRFTISRDNAKNTLYLQMNSLR PEDTAVYYCAFITTESDYDLGRRYWGQGTLVTVSS
>Nb4.2	18	EVQLVESGGGLVQPGGSLRLSCAASGSVFKINVMAWYRQAPG KGRELVAGIISGGSTSYADSVKGRFTISRDNAKNTLYLQMNSLR PEDTAVYYCAFITTESDYDLGRRYWGQGTLVTVSSGGGGSGG GSRDWDFDVFGGGTPVGG
>Nb6.1	19	EVQLVESGGGLVQPGGSLRLSCIASGLPFSTKSMGWFRQAPGK EREFVARISPGGTSRYYGDFVKGRFAISRDNAKNTTWLQMNSL KAEDTAVYYCASGERSTYIGSNYYRTNEYDYWGTGTQVTVSS AAAEQKLISEEDLNGAAHHHHHH
>Nb6.2	20	EVQLVESGGGLVQPGGSLRLSCIASGLPFSTKSMGWFRQAPGK EREFVARISPGGTSRYYGDFVKGRFAISRDNAKNTTWLQMNSL KAEDTAVYYCASGERSTYIGSNYYRTNEYDYWGTGTQVTVSS
>Nb6.4	21	EVQLLESGGGLVQPGGSLRLSCAASGLPFSTKSMGWFRQAPGK GREFVSRISPGGTSRYYGDFVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCASGERSTYIGSNYYRTNEYDYWGQGTLVTVSSA AAEQKLISEEDLNGAAHHHHHH

TABLE A (continued)

Name	SEQ ID NO:	Sequence
>Nb6.5	22	EVQLLESGGGLVQPGGSLRLSCAASGLPFSTKSMGWFRQAPGK GREFVSRISPGGTSRYYGDFVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCASGERSTYIGSNYYRTNEYDYWGQGTLVTVSS
Example 1C: wildtype	23	HHHHHHEVQLVESGGGLVQAGGSLRLSCAASGRTFNNYAMG WFRRAPGKEREFVAAITRSGVRSGVSAIYGDSVKDRFTISRDNA KNTLYLQMNSLKPEDTAVYTCAASAIGSGALRRFEYDYSGQGT QVTVSS
Example 1C: (A14P)	24	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASGRTFNNYAMG WFRRAPGKEREFVAAITRSGVRSGVSAIYGDSVKDRFTISRDNA KNTLYLQMNSLKPEDTAVYTCAASAIGSGALRRFEYDYSGQGT QVTVSS
Example 1C: (K83R)	25	HHHHHHEVQLVESGGGLVQAGGSLRLSCAASGRTFNNYAMG WFRRAPGKEREFVAAITRSGVRSGVSAIYGDSVKDRFTISRDNA KNTLYLQMNSLRPEDTAVYTCAASAIGSGALRRFEYDYSGQGT QVTVSS
Example 1C: (Q108L)	26	HHHHHHEVQLVESGGGLVQAGGSLRLSCAASGRTFNNYAMG WFRRAPGKEREFVAAITRSGVRSGVSAIYGDSVKDRFTISRDNA KNTLYLQMNSLKPEDTAVYTCAASAIGSGALRRFEYDYSGQGT LVTVSS
Example 1C: (A14P,K83R, Q108L)	27	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASGRTFNNYAMG WFRRAPGKEREFVAAITRSGVRSGVSAIYGDSVKDRFTISRDNA KNTLYLQMNSLRPEDTAVYTCAASAIGSGALRRFEYDYSGQGT LVTVSS
Example 1c: (A14P,R39Q, K83R,T91Y,Q 108L)	28	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASGRTFNNYAMG WFRQAPGKEREFVAAITRSGVRSGVSAIYGDSVKDRFTISRDNA KNTLYLQMNSLRPEDTAVYYCAASAIGSGALRRFEYDYSGQGT LVTVSS

TABLE A (continued)

Name	SEQ ID NO:	Sequence
Example 1C: (A14P,R39Q, K83R,T91Y,Q 108L)-1A	29	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASGRTFNNYAMG WFRQAPGKEREFVAAITRSGVRSGVSAIYGDSVKDRFTISRDNA KNTLYLQMNSLRPEDTAVYYCAASAIGSGALRRFEYDYSGQGT LVTVSSA
Example 1C: (A14P,R39Q, K83R,T91Y,Q 108L)-3A	30	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASGRTFNNYAMG WFRQAPGKEREFVAAITRSGVRSGVSAIYGDSVKDRFTISRDNA KNTLYLQMNSLRPEDTAVYYCAASAIGSGALRRFEYDYSGQGT LVTVSSAAA
Nb3.16	31	DVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGWYRHRPGEPREL VATITGGSSINYGDSVKGRFTISIDNSKNTVYLQMNSLRPEDTAVYYC NFNKYVTSRDTWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPG NSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADS VKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRM GWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQM NSLRPEDTAVYYCNFNKYVTSRDTWGQGTLVTVSS
Nb3.17	32	DVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGWYRHRPGEPREL VATITGGSSINYGDSVKGRFTISIDNSKNTVYLQMNSLRPEDTAVYYC NFNKYVTSRDTWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPG NSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADS VKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRM GWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDNSKNTVYLQM NSLRPEDTAVYYCNFNKYVTSRDTWGQGTLVTVSSA

TABLE A (continued)

Name	SEQ ID NO:	Sequence
C-terminal sequence	33	VTVSS
C-terminal sequence	34	$VTVSS(X)_n$
21-4-3, IGH consensus	35	QIQLVQSGPELKKPGETVKISCKASGYTFTAYSMHWVKQAPG KGLKWMGWINTVTGEPAYADDFKGRFAFSLETSASTAYLQIS SLKNEDTATYFCTRGLIHFYYWGQGTTLTVSSAKTTPPSVYPL APGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTF PAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKK IVPRDC
21-4-3-IGK consensus	36	DIQMTQTPSSLSASLGGRVTITCKASQDIHNFISWYQHKPGKV PRLIIHDTSTLQPGIPSRFSGSGSGRDYSFSITNLEPEDIATYYCL HYDNLLRSFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASV VCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTY SMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

Experimental Part:

Example 1: Generation of a polyclonal analytical antibody.

A polyclonal antibody (IgG fraction) that can be used as the "analytical antibody" was generated as follows:

A. Identification of suitable plasma samples for isolating the polyclonal antibody

Twenty plasma samples from healthy individuals that were never treated with an ISV were evaluated for presence of antibodies against ISV that can be used as the analytical antibody in the invention.

The ISV that was initially used in this Example was SEQ ID NO: 1. Subsequently, to confirm that the interaction is not specific for this particular ISV, but is an aspecific protein-protein interaction that may occur with a number of ISV's, the assays below were repeated with other ISV's (see paragraph C) below). As an alternative for SEQ ID NO:1, for example SEQ ID NO:2 may also be used.

The assay used was an ECL (Electrochemiluminescence) based bridging assay that used biotinylated ISV (a biotinylated variant of SEQ ID NO:1) to capture and sulfo-tagged ISV to detect anti-drug antibodies. A similar format is also used for performing ADA assays. Biotinylation and sulfo-tagging of the ISV was done using standard coupling chemistry on primary amines using Sulfo-NHS-LC-Biotin (Pierce) and Sulfo-tag NHS-Ester (MSD), respectively according to the manufacturer's instructions. The plasma samples were diluted 1/5 in PBS/0.1% casein and were incubated for 30 minutes at 37°C, 600 RPM in 96 well polypropylene plates. The samples (50 µL) were then diluted 1/3 in 1:1 mixture (100 µL) of 2 µg/ml biotinylated and 2 µg/ml sulfo-tagged ISV (SEQ ID NO:1) and incubated for 1 hour at RT, 600 RPM. MSD MA®96-well Standard Streptavidin plates were blocked with 150 µL/well Superblock® T20 for 1 hour at RT, then washed 3 times with PBS/0.05%Tween20 (= wash buffer). Sample/ 1:1 mix (biotinylated and sulfo-tagged ISV (SEQ ID NO:1) (50.0 µL) was transferred from the polypropylene plate to the MSD plate and incubated for 1 hour at RT, 600 rpm. Plates were washed three times prior to addition of 2 x Read Buffer (MSD) (150 µL/well) and reading the ECL units (ECLU) on an MSD instrument (Sector Imager 2400 reader). Samples were screened as positive or negative using the screening cut-point determined during method validation. The screening cut-point was calculated based on the background values of 118 individual plasma samples from healthy individuals that were

never treated with an ISV, using appropriate statistical analysis as recommended by the guidelines for ADA assay development (Shankar, 2008). A non-parametric assessment was used and the cut-off value was calculated based on the 95th percentile, after exclusion of outliers.

Six plasma samples were clearly scored as positive: IHuP#002-001-ABL-01, IHuP#002-001-ABL-08, IHuP#002-001-ABL-10, IHuP#002-001-ABL-15, IHuP#002-001-ABL-19 and IHuP#002-001-ABL-20 (Table I).

These samples were further analyzed in a drug displacement set-up (confirmatory assay) to confirm the specificity of the positive screening outcome (Table II). Therefore, the samples were diluted 1/5 in PBS/0.1%casein containing 12.5 µg/mL ISV (SEQ ID NO:1) and were incubated for 30 minutes at 37°C, 600 RPM in 96 well polypropylene plates. The samples (50 µL) are then diluted 1/3 in 1:1 mixture (100 µL) of 2 µg/ml biotinylated and 2 µg/ml sulfo-tagged ISV (SEQ ID NO:1) and incubated for 1 hour at RT, 600 RPM. Subsequently, sample/ 1: 1 mix (biotinylated and sulfo-tagged ISV) (50.0 µL) was transferred from the polypropylene plate to the blocked MSD MA®96-well Standard Streptavidin plate as described above for the screening assay and incubated for 1 hour at RT, 600 rpm. Plates were washed three times prior to addition of 2 x Read Buffer (MSD) (150 µL/well) and measuring ECL units (ECLU) on an MSD instrument (Sector Imager 2400 reader). Samples were confirmed as true positives using the confirmatory cut-point determined during method validation and was calculated on the ECL response of 118 individual plasma samples from healthy individuals that were never treated with ISV, that were spiked with 50 µg/ml ISV (SEQ ID NO:1) using appropriate statistical analysis as recommended by the guidelines for ADA assay development (Shankar, 2008). A minimal signal reduction of 50% was calculated based on the 99% confidence interval.

Samples that were positive in the ECL based bridging assay and that were confirmed as positive in the drug displacement set-up assay were selected as a source for generating the polyclonal antibody using affinity chromatography.

Table I: screening results of 20 plasma samples in the ADA ISV assay.

Sample ID	ECLU screening assay
IHuP#002-001-ABL-01	13081
IHuP#002-001-ABL-02	56
IHuP#002-001-ABL-03	272
IHuP#002-001-ABL-04	125
IHuP#002-001-ABL-05	70
IHuP#002-001-ABL-06	99
IHuP#002-001-ABL-07	170
IHuP#002-001-ABL-08	659358
IHuP#002-001-ABL-09	798
IHuP#002-001-ABL-10	1101
IHuP#002-001-ABL-11	83
IHuP#002-001-ABL-12	72
IHuP#002-001-ABL-13	403
IHuP#002-001-ABL-14	62
IHuP#002-001-ABL-15	1141
IHuP#002-001-ABL-16	159
IHuP#002-001-ABL-17	72
IHuP#002-001-ABL-18	170
IHuP#002-001-ABL-19	4503
IHuP#002-001-ABL-20	8243

Table II: Confirmation of positively screened plasma samples in the confirmatory assay. A confirmatory cut-point of 50% was used for evaluation of the results. One sample was not confirmed as a true positive sample

Plasma sample ID	ECLU screening assay: plasma	ECLU confirmatory assay: plasma	% signal inhibition
IHuP#002-001-ABL-01	13081	685	95
IHuP#002-001-ABL-08	659358	169410	74
IHuP#002-001-ABL-10	1101	582	47
IHuP#002-001-ABL-15	1141	467	59
IHuP#002-001-ABL-19	4503	1531	66
IHuP#002-001-ABL-20	8243	1450	82

A further three serum samples from individuals that not have been treated with an ISV were also evaluated using the ECL based bridging assay described above and confirmed using the drug displacement set-up assay.

Two serum samples were clearly scored as positive in the ECL based bridging assay: IHUS#B09032311A3 and IHUS#B09032311A20 (Table III). The 2 positively screened samples were further analyzed in the drug displacement set-up to confirm the specificity of the positive screening outcome.

Table III: Screening and confirmatory results of 3 serum samples and corresponding IgG purified fraction

Serum sample ID	ECL signal screening assay: serum	ECL signal confirmatory assay: serum	% signal inhibition	ECL signal screening assay: IgG	ECL signal confirmatory assay: IgG	% signal inhibition
IHUS#B09032311A3	2388	286	88%	3716	370	90%
IHUS#B09032311A20	19272	915	95%	31309	1160	96%
IHUS#B09032311A1	62					

B. Generation of purified polyclonal IgG fraction.

A polyclonal IgG was purified from the samples IHUS#B09032311A3 and IHUS#B09032311A20 (see above) using Protein G HP Spin Trap Columns (GE Healthcare) according to the manufacturer's instructions. In short, after removal of the storage solution form the column by centrifugation (30s at 100x g), the column was equilibrated by adding binding buffer (20 mM sodium phosphate, pH 7.0). After centrifugation, the solution containing the desired polyclonal was added (max 1 mg in 600 µl) and column was incubated for 4 min while gently mixing. The column was then centrifuged and washed 2x by successive addition of binding buffer (600µl) and centrifugation. After addition of 400 µl elution buffer (0,1 M glycine-HCL, pH 2.7) and mixing by inversion, the antibody was eluted by centrifugation in 30 µl neutralization buffer (1M Tris-HCL, pH 9.0).

In order to confirm that the IgG fraction thus obtained was involved in aspecific binding to the ISV(s), the purified IgG antibody was analyzed in the ECL based bridging assay described above and confirmed using the drug displacement set-up assay used under A) above. In both samples (IHUS#B09032311A3 and IHUS#B09032311A20), purified IgG antibody was confirmed to be involved in the aspecific binding leading to a positive signal in

the assays (Table III). This confirmed that the purified polyclonal IgG could be used as an "analytical antibody", and it was used as such in (the assays of) Examples 3 and 5.

C. Aspecific binding to other ISV's.

In order to determine whether the protein interference observed is specific for a single ISV, and/or is specific for a particular region, epitope or antigenic determinant on ISV's, and/or for certain mutations made to wildtype ISV's (such as one or more humanizing mutations), the ECL based bridging assay and the drug displacement set-up assay (both as described under A) above, with SEQ ID NO: 1 being used as the sulfo-tagged ISV) were repeated using the plasma samples IHUS#B09032311A3, IHUS#B09032311A20 and IHUS#B09032311A1. As these plasma samples contain the polyclonal "analytical" antibody isolated under B) above, this also provides information on the specificity, selectivity and epitope recognition of the polyclonal analytical antibody.

8 ISV's were tested (SEQ ID NO's 23 to 30, respectively – see Table A above), of which one was a wildtype VHH (SEQ ID NO: 23) and the other 7 ISV's were humanized versions of the wildtype sequence with different humanizing substitutions. Two ISV's (SEQ ID NO's: 29 and 30) also contained additional amino acid residues at the C-terminus (1 and 3 additional alanine residues, respectively).

The data are shown in Table IV. Without being limited to any explanation or hypothesis, it can be seen that changes to the C-terminal region (as defined herein) can apparently strongly influence the extent to which the plasma samples used can give rise to protein interference. For example, it can be seen that adding one or three amino acid residues to the C-terminus can strongly reduce the tendency for protein interference to arise (for example, only 18 and 13% reduction in the ECLU assay with sample IHUS#B09032311A3 for SEQ ID NO's: 29 and 30, compared to 90% reduction for SEQ ID NO: 28, the corresponding humanized variant without any amino acid residues added to the C-terminus). Similarly, introducing a proline residue at position 14 of the wildtype sequence can apparently also strongly influence the extent to which the plasma samples used can give rise to protein interference (for example, only 20 % reduction in the ECLU assay with sample IHUS#B09032311A3 for the wildtype sequence of SEQ ID NO's: 23, compared to 91% reduction for SEQ ID NO: 24, the wildtype sequence with an A14P substitution). K83R and Q108L, which are also substitutions close to the C-terminal region, also lead to some increase in the tendency to give rise to protein interference, but not as much as the A14P substitution,

and the total combined effect of the A14P+K83R+Q108L substitutions can be negated by adding one or more amino acid residues to the C-terminus (compare again the data for SEQ ID NO's: 29 and 30 with the data for the other humanized variants).

Based on this data, it was also concluded that apparently, the polyclonal analytical antibody recognized the C-terminal region (as defined herein) of ISV's generally. As can be seen from Figure 2, position 14 (and to a lesser degree positions 83 and 108) also form parts of the C-terminal region of an ISV(when the three-dimensional ternary structure of an ISV is taken into account).

Table IV: Evaluation of different Nanobody variants as competitor in the ISV ADA assay using the analytical antibody.

Serum sample ID		IHUS#B09032311 A3		IHUS#B09032311 A20		IHUS#B09032311 A1	
ECLU in screening assay (using SEQ ID NO: 1)		2217		18494		62	
Nanobody Variant (right hand column mentions the humanizing substitutions and C-terminal additions made compared to the wildtype sequence of SEQ ID NO:23		ECLU confirmatory assay	% reduction	ECLU confirmatory assay	% reduction	ECLU confirmatory assay	% reduction
SEQ ID NO: 23	Wildtype VHH	1778	20	8682	53	60	4
SEQ ID NO: 24	Wildtype VHH +A14P	205	91	668	96	56	10
SEQ ID NO: 25	Wildtype VHH +K83R	1403	37	6912	63	62	1
SEQ ID NO: 26	Wildtype VHH +Q108L	1533	31	6991	62	59	5

Table IV (continued)

Serum sample ID		IHUS#B09032311 A3		IHUS#B09032311 A20		IHUS#B09032311 A1	
ECLU in screening assay (using SEQ ID NO: 1)		2217		18494		62	
Nanobody Variant (right hand column mentions the humanizing substitutions and C-terminal additions made compared to the wildtype sequence of SEQ ID NO:23		ECLU confirmatory assay	% reduction	ECLU confirmatory assay	% reduction	ECLU confirmatory assay	% reduction
SEQ ID NO: 27	Wildtype VHH +A14P+K83R+Q108L	156	93	628	97	57	8
SEQ ID NO: 28	Wildtype VHH + A14P + R39Q + K83R + T91Y + Q108L	228	90	570	97	58	6
SEQ ID NO: 29	Wildtype VHH + A14P + R39Q + K83R + T91Y + Q108L + 1 additional A at C-terminus (A114)	1814	18	15087	18	60	3
SEQ ID NO: 30	Wildtype VHH + A14P + R39Q + K83R + T91Y + Q108L + 3 A's at C-terminus (A114+A115+A116)	1933	13	15244	18	62	0

Example 2: affinity purification of analytical antibody

This Example describes two methods that can be used to isolate from a biological fluid from a human subject an analytical antibody that is able to recognize and/or bind the C-terminal end of an ISV. The antibody is isolated from 4 different serum samples that were characterized in that these induced a positive signal in an ADA assay according to the test as described in Example 1.

Starting from serum samples, each of these protocols provide a purified preparation of interference factor(s) that can be used as the analytical antibody in the methods described herein. These methods can also more generally be used to purify the interference factor(s) for other purposes (for example, the interference factor(s) purified using the protocols below were also used experimentally in Example 8 in order to show that binding to an ISV or ISV-construct by monoclonal 21-4 is predictive for binding of the same ISV or ISV-construct by interference factors, and thus by the tendency of said ISV or ISV-construct to undergo aspecific protein interference in an ADA assay).

Example 2A: purification using protein A and affinity chromatography

In a first step, the IgG antibody fraction was enriched from the serum samples using protein A affinity chromatography. Typical columns that were used for this enrichment included HiTrap MabselectSure and MabSelectXtra (GE Healthcare); PorosMabCapture A (Applied Biosystems). Purification of the IgG antibodies from the serum samples was performed in an automated and similar manner over all experiments. Chromatographic runs were performed on the AKTA purifier systems (GE Healthcare) and logged in real-time using UNICORN protein purification software (GE Healthcare). Briefly, the serum sample was diluted 1:1 with D-PBS (Dulbecco's Phosphate-Buffered Saline) and 0.22 µm filtered before uploading on the column at a fixed flow rate of 0.5 mL/ min. The column was washed to remove non-specific binding components over 5 column volumes using D-PBS at a flow rate of 0.5 mL/min. The IgG fraction was eluted by acidic elution, using 100 mM Glycine pH 2.6 buffer, and a flow rate of 0.5 mL/min. After elution, the fractions were neutralized using 1.5 M Tris buffer pH 8.8. SDS-PAGE was run to confirm the isolation of IgG antibodies in the elution.

In a second step, the interfering IgGs were further enriched by applying the protein A purified IgG fraction from the 4 different sera onto ISV coupled affinity columns. More specifically, the interfering IgG were further enriched by binding to a column containing an ISV with sequence of SEQ ID NO: 1. To this, the ISV was covalently linked to Sepharose 4 fast flow (GE Healthcare) using the CNBr (Cyanogen bromide) -coupling method according to the manufacturer's procedure. The affinity purification was performed in an automated and similar manner over all experiments. Chromatographic runs were performed on the AKTA purifier systems and logged in UNICORN. Briefly, the IgG enriched sample (up to 10mL loading volume) was uploaded on the column at a fixed flow rate of 0.5 mL/ min. The column was washed to remove non-specific binding-components over 5 column volumes using D-PBS at a flow rate of 0.5 mL/min. The ISV-binding components were eluted by acidic elution, using 100 mM Glycine pH 2.6 buffer, and a flow rate of 0.5 mL/min. After elution, the fractions were neutralized using 1.5 M Tris buffer pH 8.8. The fractions were analyzed using SDS-PAGE which confirmed the isolation of IgG antibodies in the elution (data not shown).

These fractions were pooled and used for further analyses such as those described in Example 3.

Example 2B: purification suing CaptureSelect™ chromatography.

Alternatively, interference factor(s) were recovered from plasma and purified using the commercially available IgA binding affinity resin CaptureSelect hIgA™ (BAC BV), which is based on camelid-derived heavy-chain only variable domains (VHH). The collected 'IgA fraction' containing IgA together with interfering IgG was subsequently loaded onto a protein A column to remove the IgA fraction. The protein A column was processed according to generic IgG purification conditions (running buffer: PBS; elution buffer: 100mM glycine pH=2.7; post elution neutralization via 1M Tris). The interference factor was recovered from the Prot A elution in >95% yield.

In a variation to this method, another CaptureSelect affinity resin (CaptureSelect Alpha-1 Antitrypsin resin, a VHH based commercially available affinity resin, not targeting any antibody related proteins) was be used. This resin provided a high interference factor binding efficacy and allowed for a selective 2 step elution: antitrypsin via neutral pH elution using 2.0 M MgCl2, followed by the interference factor elution via an acidic step (0.1 M

Glycine pH3.0, similar to protein A/G elution conditions; neutralisation using 1.5M Tris). This one step purification yielded up to 15 µg interfering IgG1 per mL high interference plasma, which is approximately 0.3% of the total IgG present. Optionally, the neutralised interference fraction can be desalted and further purified via a Size Exclusion Column equilibrated in D-PBS.

Example 3: Influence of different ISV substitutions on the tendency of ISV to give rise to protein interference

As mentioned in the description above, the present invention makes available certain assays and techniques which make it possible to make an assessment of whether or not a given ISV has a tendency to give rise to protein interference. These include the ECL based bridging assay and the drug displacement set-up assay used in Example 1, as well as the BIACORE assay described in this Example 3 and the bridging/competition ADA assay described in the further Examples below.

As also mentioned in the description above, these assays can also be used to determine whether specific changes (such as amino acid deletions, substitutions or additions) can influence (and preferably reduce) the tendency of a given ISV to give rise to protein interference. Some of these changes will be or become clear to the skilled person based on the disclosure herein and on the experimental data presented in Example 1 and this Example 3.

As already indicated by the data generated in Example 1, it appears that certain mutations in or close to the C-terminal region (as defined herein) of an ISV can (strongly) influence its tendency to give rise to protein interference. For example, adding a few amino acid residues to the C-terminus (such as 1 or 3 alanine residues) appears to strongly reduce the tendency of an ISV to give rise to protein interference, and appears even to be able to negate the presence of other substitutions (for example, in or close to the C-terminal region) which appear to increase the tendency to give rise to protein interference (for example, an A14P substitution).

In this Example 3, both the effect of other substitutions as well as the effect of adding additional amino acids to the C-terminus was investigated by comparing related ISV's with different substitutions, using the analytical polyclonal antibody generated in Example 2. The analysis was done by measuring the kinetics of interaction between each of the ISV's

investigated and the analytical polyclonal by means of surface Plasmon resonance (SPR) using the Biacore™ T100 biosensor from GE Healthcare. The ISV tested in this Example 3 were those of SEQ ID NO's 3 to 22 (see Table A above and Table V below).

In a typical experiment, a polyclonal antibody solution was prepared at 10 µg/ml in 10 mM NaOAc pH5.0. This polyclonal antibody was then immobilized on a CM5 sensorchip using amine coupling by the EDC/NHS method (EDC=N-ethyl-N'-[3-diethylamino-propyl]-carbodiimide; NHS=N-hydroxysuccinimide) according to the manufacturer's procedure. The amount immobilized gave approximately 2700 response units (RU). A fixed concentration of 500 nM of ISV was then injected onto the surface for 120 seconds at a flow rate of 45 µl per minute. Because no efficient regeneration buffer could be identified, the dissociation time was elongated to 2400 seconds. The signal obtained by injecting the ISV onto a blank flow cell was subtracted from the signal obtained by injecting the ISV onto the polyclonal antibody bound flow cell. The blank flow cell was activated/deactivated in a similar way as the flow cell for the polyclonal antibody, but without adding protein. Also, a blank injection (HBS-EP + running buffer (HBS= Hepes Buffered Saline: GE Healthcare) was subtracted to correct for possible baseline drift.

To examine the effect of adding amino acid residues to the C-terminus, the influence of adding 1 or 2 alanines and 1, 2 or 3 glycines was investigated by comparing the binding of ISV with the different additions, using an analytical polyclonal antibody generated as described in example 2. The ISV's generated and tested for this purpose were NB's 3.4 to 3.9 (SEQ ID NO's: 5 to 10).

As representative examples of the kind of data obtained, Figure 3 shows the binding of NB's 3.4 to 3.9 to the immobilized polyclonal antibody. Table V summarizes the results obtained.

TABLE V

Clone ID	SEQ ID NO	Position 113(1)	Position 114(1)	Position 115(1)	Position 116(1)	Binding** (RU)
NB 3.4	**5**	S				75
NB 3.5	**6**	S	A			9
NB 3.6	**7**	S	A			8
				A		
NB 3.7	**8**	S	G			31
NB 3.8	**9**	S	G	G		13
NB 3.9	**10**	S	G	G	G	13

**: Binding signal obtained at the end of injection (=maximal RU signal)

(1) In this numbering, position 113 is the last "S" of the C-terminal VTVSS motif, and positions 114, 115 and 116 are the positions immediately following (downstream) of said position 113.

To examine the effect of (other) substitutions in the C-terminal region, the influence of different substitutions was investigated by comparing related ISV's containing these substitutions, using the same analytical polyclonal antibody as described above. The analysis was done as described above.

The ISVs containing said substitutions that were tested were NB's 3.1, 3.2 and 3.4 (SEQ ID NO's 3, 4 and 5); NB's 3.10 to 3.15 (SEQ ID NO's 11 to 16), which were compared with NB 3.4; NB's 4.1 and 4.2 (SEQ ID NO's 17 and 18) and NB's 6.1, 6.2, 6.4 and 6.5 (SEQ ID NO's 19 to 22).

As representative examples of the kind of data obtained:

- Figure 4 shows the binding of NB's 3.4, 3.11, 3.12 and 3.13 to the immobilized polyclonal antibody;
- Figure 5 shows the binding of NB's 3.4, 3.14 and 3.15 to the immobilized polyclonal antibody;
- Figure 6 shows the binding of NB's 3.1, 3.2 and 3.4 to the immobilized polyclonal antibody;
- Figure 7 shows the binding of NB's 4.1 and 4.2 to the immobilized polyclonal antibody;

- Figure 8 shows the binding of NB's 6.1, 6.2, 6.4 and 6.5 to the immobilized polyclonal antibody.

Tables VI, VII and VIII summarize the results obtained.

TABLE VI

Clone ID	SEQ ID NO	Position 14(1)	Position 83(1)	Position 108(1)	Binding** (RU)
NB 3.4	5	P	R	L	75
NB 3.10	11	A	R	L	91
NB 3.11	12	P	K	L	88
NB 3.12	13	A	R	Q	86
NB 3.13	14	P	R	Q	90

**: Binding signal obtained at the end of injection (=maximal RU signal)

(1) numbering according to Kabat.

TABLE VII

Clone ID	SEQ ID NO	Position 11(1)	Position 110(1)	Binding** (RU)
NB 3.4	5	L	T	75
NB 3.14	15	L	Q	79
NB 3.15	16	S	T	22

**: Binding signal obtained at the end of injection (=maximal RU signal)

(1) numbering according to Kabat.

TABLE VIII

Clone ID	**SEQ ID NO:**	**Position 14**	**Position 83[(1)]**	**Position 108 [(2)]**	**Tag***	**Binding** (RU)**
NB 3.1	3	A	K	Q	-	2
NB 3.2	4	A	K	Q	+	0
NB 3.4	5	P	R	L	-	59
Clone ID		**Position 14**	**Position 83 [(3)]**	**Position 108 [(4)]**	**Tag***	**Binding** (RU)**
NB 4.1	17	P	R	L	-	51
NB 4.2	18	P	R	L	+	0
Clone ID		**Position 14**	**Position 83 [(5)]**	**Position 108 [(6)]**	**Tag***	**Binding** (RU)**
NB 6.1	19	P	K	Q	+	0
NB 6.2	20	P	K	Q	-	39
NB 6.4	21	P	R	L	+	0
NB 6.5	22	P	R	L	-	66

*: if "+", this ISV contains additional amino acids at the C-terminal VTVSS end

**: Binding signal obtained at the end of injection (=maximal RU signal)

[(1)]: numbering acc. to Kabat (corresponds to the a.a. at position 87 in SEQ ID NO's 3 to 5).

[(2)]: numbering acc. to Kabat (corresponds to the a.a. at position 123 in SEQ ID NO's 3 to 5).

[(3)]: numbering acc. to Kabat (corresponds to the a.a. at position 86 in SEQ ID NO's 17 and 18).

[(4)]: numbering acc. to Kabat (corresponds to the a.a. at position 116 in SEQ ID NO's 17 and 18).

[(5)]: numbering acc. to Kabat (corresponds to the a.a. at position 86 in SEQ ID NO's 19 to 22).

[(6)]: numbering acc. to Kabat (corresponds to the a.a. at position 112 in SEQ ID NO's 19 to 22).

Again, without being limited to any specific hypothesis or explanation, the data presented above shows that (various) substitutions to the C-terminal region (as defined herein) of an ISV can alter/improve its tendency to give rise to protein interference.

Example 4: representative protocols for performing the ADA assays of Figure 1.

This Example gives some representative but non-limiting conditions that could be used for performing the competitive/bridging ADA assays schematically shown in Figure 1:

- ADA assay of Figure 1A in solution: Samples 100% matrix, 30', 37°C, Acid treatment using acetic acid in 10 matrix, 5', RT, Preincubation/acid neutralisation sample: ISV-Sulfo (:Tris) 1:1:1 (1: 0,9:0,9: 0,1), 1h, RT; On plate 1 h, RT; Wash 3x, Readbuffer 4X
- ADA assay of Figure 1B in solution: Samples 20% matrix, 30', 37°C, Preincubation sample: ISV- -Sulfo 1:1:1, 1h, RT, On plate 1 h, RT, Wash 3x, Readbuffer 2x
- Sequential ADA assay of Figure 1C: Capture ISV-Bio, 1 h, RT, Wash 3X, Samples 20% matrix, 15', RT, On plate: 2h, RT, Wash 3X, Detection ALX-0141-Sulfo, 1h, RT, Wash 3x, Readbuffer 4X

Example 5: Predicting sensitivity of the ISV to aspecific protein interference using the analytical antibody.

This example describes a bridging/competition ADA assay using the analytical antibody that can be used to predict sensitivity of an ISV to aspecific protein interference.

The ISV to be tested is diluted at a concentration of 10 µg/ml and incubated with the analytical antibody at 400 ng/ml, purified according to Example 2, and incubated at 37°C at 600 rpm in 96 well polypropylene plates. The sample (50 µL) is then diluted 1/3 in 1:1 mixture (100 µL) of 2 µg/ml biotinylated and 2 µg/ml sulfo-tagged ISV and incubated for 1 hour at RT, 600 RPM. MSD MA®96-well Standard Streptavidin plates are blocked with 150 µL/well Superblock® T20 for 1 hour at RT, then washed 3 times with PBS/0.05%Tween20 (= wash buffer). Sample/ 1:1 mix (biotinylated and sulfo-tagged ISV) (50.0 µL) is transferred from the polypropylene plate to the MSD plate and incubated for 1 hour at RT, 600 rpm. Plates are washed three times prior to addition of 2 x Read Buffer (MSD) (150 µL/well) and reading the ECL units (ECLU) on an MSD instrument (Sector Imager 2400 reader).

Using this assay, the ISVs of SEQ ID NO's 23 to 30 were tested and compared. The data are shown in Table IX. These data not only show that the assay described in this Example can be used to predict the tendency of an ISV to give rise to protein interference, but the data generated also confirm the findings from the earlier Examples on the effect of

substitutions in the C-terminal region. As can be seen, addition of 3 (and to lesser extent 1) Alanine residues at the C-terminus of the fully humanized ISV abolished its capacity to compete with binding of the analytical antibody. Mutating position 14 on the wild type ISV variant from Alanine to Proline clearly increased its capacity as competitor in the assay, (=making the ISV variant more prone to aspecific protein interference), whereas mutating position 83 and 108 did not clearly influenced the sensitivity of the ISV to aspecific protein interference.

Table IX

ID affinity purified antibody		IHuP#002-001-ABL-08	
ECLU in screening assay (using SEQ ID NO:1)		2919	
Nanobody Variant (right hand column mentions the humanizing substitutions and C-terminal additions made compared to the wildtype sequence of SEQ ID NO: 23)		**ECLU confirmatory assay**	**% reduction**
SEQ ID NO: 23	Wildtype VHH	2706	7.3
SEQ ID NO: 24	Wildtype VHH +A14P	268	90.8
SEQ ID NO: 25	Wildtype VHH +K83R	2460	15.71
SEQ ID NO: 26	Wildtype VHH +Q108L	2533	13.23
SEQ ID NO: 27	Wildtype VHH +A14P+K83R+Q108L	319	89.1
SEQ ID NO: 28	Wildtype VHH + A14P + R39Q + K83R + T91Y + Q108L	251	91.4
SEQ ID NO: 29	Wildtype VHH + A14P + R39Q + K83R + T91Y + Q108L + 1 additional A at C-terminus (A114)	1207	58.64
SEQ ID NO: 30	Wildtype VHH + A14P + R39Q + K83R + T91Y + Q108L + 3 A's at C-terminus (A114+A115+A116)	3301	-13.09

Example 6: Influence of the addition of amino acids to the C-terminus of anti-OX40L Nanobodies on their OX40L blocking potency.

This example demonstrates that the C-terminal extension has no influence on activity or blocking potency of the Nanobodies.

The *in vitro* potency of the trivalent bispecific sequence optimized anti-OX40L Nanobody Nb 3.16 (SEQ ID NO: 31) was compared with the potency of the corresponding Nanobody containing one additional Ala at its C-terminus Nb 3.17 (SEQ ID NO: 32).

A first assay, the T-cell activation assay, was performed as follows. PBMCs were isolated from buffy coats (Red Cross, Ghent, Belgium) from healthy donors using Ficoll Paque Plus reagent (GE Healthcare) and washed using RPMI 1640 complete medium (RPMI1640 + GlutaMAX + 25 mM HEPES + 10% fetal bovine serum + 1% Penicillin/Streptomycin; Invitrogen). The PBMC's ($1x10^5$ cells/well) were stimulated with phytohaemagglutinin (PHA-L; final concentration 0.6µg/ml) before the addition to $1x10^4$ hOX40L expressing CHO cells (irradiated with gamma scintillator at 3000 RAD; UZ Gent, Belgium) and dilution series of anti-OX40L Nanobodies RPMI 1640 complete medium and incubated for 22 hours at 37°C in CO_2 incubator. Production of IL2 by the PBMCs was measured in ELISA. Wells of a Maxisorp plate were coated overnight at 4°C with anti-human IL2 monoclonal antibody (BD Biosciences). After washing and blocking of the coated wells, a ½ dilution of cell supernatant was added. As a standard, ½ serial dilutions of recombinant human IL2 (BD Biosciences) starting from 2000 pg/ml were included. Detection was done using biotinylated anti-human IL2 monoclonal antibody (BD Biosciences) and HRP conjugated streptavidin (Thermo Scientific) and esTMB (SDT Reagents). The reaction was stopped with 1N HCl and the OD was read at 450 nm. As expected, the potency of the trivalent bispecific sequence optimized Nanobody Nb 3.17 (IC50 = 0.13nM, 95% CI = 0.098-0.17nM) was comparable to that of Nb 3.16 (IC50= 0.10nM, 95% CI = 0.071-0.15 nM).

In a second ELISA-based competition assay, a dilution series (from 1.5µM to 0.083 pM) of the Nanobodies were pre-incubated overnight at room temperature with 100ng/ml human OX40/Fc (R&D Systems) and 10ng/ml biotinylated human OX40L (R&D Systems; in-house biotinylated as described in Example 1) in PBS +0.1% BSA +0.01% Tween-20. Next, the samples were incubated on Maxisorp plates coated with 10ug/ml anti-human Fc Nanobody (in-house generated) and blocked with PBS + 1% BSA +0.1% Tween-20. Bound human OX40/Fc was detected using HRP conjugated streptavidin (Thermo Scientific) and sTMB (SDT Reagents). The reaction was stopped with 1N HCl and the OD was read at 450 nm. In accordance with the cell-based assay, the potency of the trivalent bispecific sequence optimized Nanobodies Nb 3.17 (IC50= 0.178nM, 95% CI = 0.152-0.200nM) was comparable to that of Nb 3.16 (IC50 = 0.179nM, 95% CI = 0.149-0.215nM).

Example 7: generation of monoclonal antibody 21-4-3.

Two groups of different mice strains (BALB/c and NMRI - three mice each) were intraperitoneally immunized with the Nanobody construct of SEQ ID NO:98 in WO 2006/122825, in a water-in-oil emulsion of equal volumes of antigen and Freund´s complete or incomplete adjuvant) over a period of 39 days, with boosting until suitable antiserum titers were obtained.

After asphyxiation of the stimulated mice in CO2, the spleens were aseptically removed and a single cell suspension of pooled spleens was prepared. Spleen cells and myeloma cells were washed several times with DMEM and fused in the presence of 1 ml 50% (w/v) PEG 3350 (ratio spleen cells to SP2/0 3:1). For fusion was used the myeloma cell line SP2/0-Ag14 from German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig). This cell line is a hybrid between BALB/c spleen cells and the myeloma cell line P3x63Ag8. The so produced hybridomas were resuspended in CGM containing 20% FCS and aminopterin (HAT medium) and plated out (140 µl/well) into eight 96-well tissue culture flat-bottom plates (Corning-Costar) containing 140 µl/well CGM (20% FCS) with peritoneal excudate cells as feeder cells. The plates were incubated for 10 days in a complete growth medium (CGM) containing DMEM with supplements 2-mercaptoethanol, L-Glutamin, Stable Glutamin, HT and non essential amino acids (in concentrations recommended by the supplier) and FCS at different concentrations (10%, 15% or 20%). During this period cells were fed two times with HAT medium. The cell culture supernatants from hybridoma cells usually contained 1 to 20 µg/ml antibody, which were tested in a binding ELISA to confirm binding to the Nanobody construct of SEQ ID NO:98 in WO 2006/122825.

Cells from positive IgG producing wells were transferred into wells of 48 well plates and cultivated for 2- 4 days (depending on growth characteristic of cells). Binding ELISA's on ALX081 and human/cynomolgus IgG were carried out in order to exclude the unspecific binders. Hybridoma cells expressing binders specific for the Nanobody construct of SEQ ID NO:98 in WO 2006/122825 were twice cloned using limited dilution. After fusion and rescreening 7 primary cultures producing antibodies against ALX-081 were identified. All these primary cultures produced antibodies not cross-reacting with human or cynomolgus IgG. The primary cultures were recloned (twice).

Clone 21-4 (one of the clones that stably produced antibodies against ALX-081 after the second cloning) was given the designation "ABH0015" and was deposited with the Belgian Coordinated Collections of Micro-organisms (BCCM) in Ghent, Belgium on June 4, 2012 under accession number LMBP-9680-CB. The mouse monoclonal produced by ABH0015 was called 21-4-3: isotype determination for 21-4-3 showed an IgG1 heavy chain and a kappa light chain, which were sequenced (see SEQ ID NO's: 35 and 36, respectively). 21-4-3 was shown to bind to the C-terminal region of the Nanobody construct of SEQ ID NO:98 in WO 2006/122825 (data not shown).

Example 8: binding of 21-4 to an ISV is predictive of the tendency of an ISV to undergo aspecific protein interference

This Example together with the following Example 9 demonstrates that binding of the monoclonal 21-4 to an ISV can be used to predict (within the degrees of certainty indicated in this Example) of whether a given ISV will have a tendency to undergo aspecific protein interference (e.g. in an ADA assay).

This Example 8 in particular shows that 21-4 can be used to predict whether certain proposed modifications to a given ISV (such as adding one or more amino acid residues to the C-terminus of an ISV and/or substituting one or more amino acid substitutions within the C-terminal region of an ISV) will lead to a reduction of the tendency of said ISV to undergo aspecific protein interference.

In short, a set of 53 different Nanobodies and Nanobody constructs (see Figure 9 and SEQ ID NO's: 38 to 89) were tested for binding by monoclonal 21-4-3. The same Nanobodies and Nanobody constructs were also tested for binding by purified preparations of interference factor(s) obtained from three different human donors (referred to herein as "Donor 8", "Donor 19" and "Donor 30"), to see if there was any correlation between binding by 21-4 and by the purified interference factors.

It was established that binding of an ISV by 21-4 can indeed be used to predict binding of the same ISV's by the interference factor(s) (within the overall degree of confidence provided by the data set out herein).

To demonstrate this, as detailed by the experimental data set out below, the binding of the 53 Nanobodies or Nanobody constructs (as listed in Figure 9; see SEQ ID NO's: 38 to 89) by 21-4 was measured using a Biacore T100 (according to the protocol set out below) and

was compared to binding of a reference Nanobody or construct (also listed in Figure 9), as measured using the same Biacore instrument and the same protocol. The results are shown in Table X below.

Table X

SEQ ID NO:	C-terminal amino acid(s)	mutations to the C-terminal region	reduction in binding of 21-4-3 vs binding of Reference Sequence (=100 %)	reduction of interference in serum from Donor A compared to Reference Sequence (=100%)	reduction of interference in serum from Donor B compared to Reference Sequence (=100%)	reduction of interference in serum from Donor C compared to Reference Sequence (=100%)	More than 70% reduction in binding of Nanobody to 21-4-3 predicts >50% reduction in binding of nanobody to interference	More than 90% reduction in binding of Nanobody to 21-4-3 predicts >50% reduction in binding of nanobody to interference
37	A	none	7%	3%	21%	31%	ok	ok
38	A	none	0%	9%	25%	7%	ok	ok
39	A	none	0%	10%	43%	35%	ok	ok
40	A	none	1%	6%	#N/A	#N/A	ok	ok
41	A	none	7%	6%	9%	#N/A	ok	ok
42	A	none	0%	1%	4%	#N/A	ok	ok
43	A	none	3%	3%	20%	#N/A	ok	ok
44	A	none	1%	5%	#N/A	#N/A	ok	ok

Table X (continued – see column headers above)

45	none	P14A, P41T, S62F, S74A, S82bN, R83K, L108Q	22%	0%	#N/A	ok	
46	AAEQKLI SEEDLN GAAHHH HHH	A14P, T41P, F62S, A74S, N82bS, K83R, Q108L	2%	0%	#N/A	ok	ok
47	GGGGSG GGSRDW DFDVFG GGTPVG G	none	4%	1%	#N/A	ok	ok
48	AAEQKLI SEEDLN GAAHHH HHH	none	3%	0%	#N/A	ok	ok

Table X (continued – see column headers above)

49	AAEQKLI SEEDLN GAAHHH HHH	V5L, I23A, E44G, A49S, A68T, A74S, T78L, W79Y, K83R, T110Q, Q108L	4%	0%	#N/A	#N/A	OK	OK
50	none	L11S	44%	77%	33%	#N/A	(<70% reduction)	(<90% reduction)
51	none	T110Q	88%	85%	84%	#N/A	(<70% reduction)	(<90% reduction)
52	none	S112G	100%	84%	58%	#N/A	(<70% reduction)	(<90% reduction)
53	none	S113G	13%	85%	88%	#N/A	NOK	(<90% reduction)
54	none	L11S, T110Q, S112G, S113G	16%	39%	16%	#N/A	OK	OK
55	A	none	6%	2%	21%	31%	OK	OK

Table X (continued – see column headers above)

56	G	S113G	3%	2%	25%	0%	OK	OK
57	AS	none	6%	1%	2%	#N/A	OK	OK
58	AST	none	6%	2%	2%	#N/A	OK	OK
59	ASTK	none	6%	2%	1%	#N/A	OK	OK
60	ASP	none	6%	2%	1%	#N/A	OK	OK
61	AP	none	6%	2%	2%	#N/A	OK	OK
62	APT	none	6%	2%	1%	#N/A	OK	OK
63	W	none	3%	4%	8%	#N/A	OK	OK
64	L	none	6%	3%	4%	#N/A	OK	OK
65	none	P14A	23%	73%	121%	64%	NOK	(<90% reduction)
66	none	L11S	48%	81%	29%	84%	(<70% reduction)	(<90% reduction)
67	none	R83K	101%	102%	117%	96%	(<70% reduction)	(<90% reduction)
68	none	P14A, L108Q	26%	38%	115%	49%	NOK	(<90% reduction)

Table X (continued – see column headers above)

69	none	L108Q	106%	80%	120%	84%	(<70% reduction)	(<90% reduction)
70	none	T110Q	106%	90%	105%	98%	(<70% reduction)	(<90% reduction)
71	none	S113G	44%	88%	105%	87%	(<70% reduction)	(<90% reduction)
72	none	S112G, S113G	45%	70%	47%	56%	(<70% reduction)	(<90% reduction)
73	G	S112G, S113G	1%	6%	8%	9%	OK	OK
74	G	none	1%	4%	41%	33%	OK	OK
75	AA	none	1%	1%	11%	16%	OK	OK
76	GGG	none	2%	2%	17%	20%	OK	OK
77	A	none	1%	2%	12%	13%	OK	OK
78	none	Q13R	1%	96%	96%	100%	NOK	NOK
79	GG	none	2%	1%	17%	13%	OK	OK
80	none	T110Q, S112G, S113G	51%	65%	5%	58%	(<70% reduction)	(<90% reduction)

Table X (continued – see column headers above)

81	none	L11V	75%	94%	50%	89%	(<70% reduction)	(<90% reduction)
82	none	P84A	56%	96%	80%	100%	(<70% reduction)	(<90% reduction)
83	none	T87A	79%	56%	73%	61%	(<70% reduction)	(<90% reduction)
84	none	S112G	91%	84%	50%	83%	(<70% reduction)	(<90% reduction)
85	none	L11S, T110Q, S112G, S113G	32%	46%	5%	42%	(<70% reduction)	(<90% reduction)
86	none	L11S, T110Q	64%	76%	6%	86%	(<70% reduction)	(<90% reduction)
87	none	L11S, S112G, S113G	41%	51%	5%	39%	(<70% reduction)	(<90% reduction)
88	A	L11S, T110Q	1%	1%	5%	11%	OK	OK
89	none	L11S, P14A, T110Q, S112G, S113G	2%	14%	5%	17%	OK	OK

For each of the 53 Nanobodies or Nanobody constructs tested, the reference was chosen such that compared to the reference, the tested Nanobodies or Nanobody constructs either had one or more additional amino acid residues at the C-terminal end (which were added in order to test the effect of such addition on protein interference, and in particular in order to reduce said interference) and/or one or more mutations within the C-terminal region (for example, as a result of humanization compared to the reference).

The results were expressed as a percentage reduction in binding (measured as RU units) for the given Nanobody versus the binding of the reference (also measured in RU units - for example, if the measured binding level (RU) of the reference Nanobody was 276 and the binding level of the given Nanobody (also in RU) was 9, then the reduction in binding level was to a level of [9 RU/276 RU] x 100% = 3%), which means a reduction of 97% compared to the reference (100%).

Similarly, binding of the purified interference factor(s) from each of the three donors to each of the 53 Nanobodies or Nanobody constructs was measured using the same Biacore instrument and compared to binding of the purified interference factor(s) to the same reference Nanobody or construct. The results were similarly expressed as a percentage reduction in binding of the interference factor to the given Nanobody or Nanobody construct vs the reference.

It was found that for essentially all Nanobody or Nanobody construct in which one or more amino acid residues had been added to the C-terminal end compared to the reference, that the binding of the interference factor(s) was dramatically reduced. This again confirms that adding one or more amino acid residues to the C-terminal end of an ISV (VTVSS) can reduce aspecific protein interference in an ADA assay. It was also found that in the majority of cases, only making substitutions within the C-terminal region (i.e. without adding one or more amino acid residues to the C-terminus) compared to the reference often did not have a similar dramatic impact on the binding of the interference factor(s).

The data was then further analysed to determine whether a reduction in binding by 21-4 compared to the reference was in any way correlated with a reduction in binding by each of the three different preparations of purified interference factor compared to the reference. Such correlations were found.

For example, it was found that of the 54 Nanobodies or Nanobody constructs tested, 36 showed a reduction in binding by 21-4 of more than 70% compared to their respective

reference sequence (with most of these 36 having one or more additional amino acid residues at the C-terminus, in some cases in combination with substitutions within the C-terminal region). Of these 36, 32 also showed reduction in binding by the interference factor(s) compared to the reference of more than 50% (and in a large number of cases, in particular for Nanobodies or Nanobody constructs with one or more amino acid residues added at the C-terminus, the reduction was far greater than 50%, such as more than 70% or even more than 90%, see the data given in the Table X). This demonstrates that in 32 out of 36 cases (i.e. 89%), a reduction in binding by 21-4 of more than 70% (compared to the reference = 100%) is predictive for a reduction in binding by the interference factors of more than 50% (compared to the same reference). For clarity, in each case, the reduction was calculated as 100% - [the percentage given in the Tables below for the level of reduction achieved with the Nanobody tested].

Similarly, it was found that of the 53 Nanobodies or Nanobody constructs tested, 33 showed a reduction in binding by 21-4 of more than 90% compared to their respective reference sequence (again, with most of these 33 having one or more additional amino acid residues at the C-terminus, in some cases in combination with substitutions within the C-terminal region). Of these 33, 32 also showed reduction in binding by the interference factor(s) compared to their respective reference sequence of more than 50%. This demonstrates that in 32 out of 33 cases (i.e. 97%), a reduction in binding by 21-4 of more than 90% (compared to the reference) is predictive for a reduction in binding by the interference factors of more than 50% (compared to the same reference).

It should also be noted that such a reduction in binding of the interference factor(s) by more than 50% (as evidenced by a reduction of binding by 21-4 of more than 70%) means that such interference factor(s) essentially no longer interfere(s) with an ADA assay for the ISV in question: experimental confirmation using an ADA assay showed that when the binding by the interference factor(s) is reduced by more than 45%, that no significant influence of the presence of the interference factor(s) on the ADA assay could be observed. In this respect, it will be also be clear to the skilled person that this will even more so be the case when the binding by interference factor(s) is reduced to an extent far greater than 50% (such as by more than 70% or even more than 90%), as is observed in some cases (see again the data presented herein).

In fact, it has been found that a reduction of more than 45% of binding by 21-4 is indicative of a reduction of binding by interference factors of more than 45%, which as mentioned means that the interference factor(s) no longer interfere with the ADA assay.

Moreover, the data presented herein on the correlation between (reduction in) binding by 21-4 and (reduction in) binding by interference factor also allowed the present inventors to set an absolute value for the binding by 21-4 below which it can be expected (within the confidence provided by the data set out in this Example 8) that an ISV or ISV-based construct will not be susceptible to binding by interference factor(s) in a way that could interfere with an ADA assay. As set out in the following Example 9, this value is 500 RU (determined and calculated as set out in Example 9).

Monoclonal 21-4 was purified from the culture medium of the hybridoma obtained in Example 7 above, as follows: Hybridoma cells secreting the monoclonal antibody 21-4-3 were cultured in spinner flasks in serum free medium (CD Hybridoma, Gibco, supplemented with 8mM L-glutamine (Invitrogen) and 1×cholesterol (250× cholersterol lipid concentrate, Gibco)) at a volume of 100mL or 500mL. The cleared supernatant was filtered, and the murine IgG1 captured on a ProteinA column (HiTrap MabSelect SuRe, 5mL, GE Healthcare) at a reduced flow rate of 2mL/min. Bound antibody was eluted in 0.1M citrate buffer pH3.0, and elution fractions (of 5mL) directly neutralized with 1mL of 1M TRIS pH9. Purity of the antibody was verified by reducing and non-reducing SDS-PAGE.

The purified preparations of interference factor(s) from Donors 8 and 19 were obtained from serum samples from said donors by means of affinity purification, essentially as described in Example 2A. The interference factor(s) from Donor 30 were obtained from a serum sample of Donor 30, essentially as described in Example 2B.

To determine the binding of 21-4 to each of the Nanobodies or Nanobody constructs, the protocol described in Example 9 was used.

The binding of the interference factors from the three donors to each of the Nanobodies or Nanobody constructs was determined using a Biacore T100 essentially as described in Example 3, using the interference factor from each of the donors 8, 19 and 30, directly immobilized on a CM5 sensor chip.

Example 9: protocol for predicting whether an ISV will have a tendency to undergo aspecific protein interference (using monoclonal 21-4).

Binding measurements were performed using a Biacore T100 using a CM5 T120416 sensor chip, with running buffer HBS-EP+, 25°C. 21-4 was captured via immobilized rabbit anti-mouse IgG, as it was found that directly immobilized mAb 21-4-3 surface could not efficiently be regenerated. The anti-mouse IgG used was a polyclonal rabbit anti-mouse IgG antibodies reacting with all IgG subclasses, IgA and IgM (GE Healthcare; Cat#BR-1008-38; Lot#10056316). Immobilisation of the anti-mouse IgG was performed using manual amine coupling using a 7 minute injection of EDC/NHS for activation and a 7 minute injection of 1M ethanolamine HCl pH 8.5 for deactivation (Biacore, amine coupling kit). Binding conditions are listed in Table XI. Based on the immobilization level and MW of the proteins, the theoretical R_{max} for mAb21-4-3 binding to the immobilized anti-mouse IgG was ~13000RU (when one mAb21-4-3 molecule is binding to one anti-mouse IgG molecule).

Table XI

Protein	**Conc. (µg/ml)**	**Contact time (s)**	**Flow rate (µl/min)**	**Immobilization buffer**	**Immobilization level (RU)**
Anti-mouse IgG	30	420	5	10mM acetate pH5.0	13028
Anti-mouse IgG	30	420 24	5	10mM acetate pH5.0	13318

The conditions used for the binding experiment (Biacore T100) using 21-4 immobilized in the manner are given in Table XII. The anti-mouse IgG surface could successfully be regenerated after capture of mAb21-4-3 and injection of all samples (with a limited increase for baseline level after each regeneration).

Table XII

Capture	
Flow path	4
Flow rate (µl/min)	10
Contact time (s)	180
Concentration (µg/ml)	10
Binding and dissociation	
Flow path	3,4
Flow rate (µl/min)	45
Sample contact time (s)	120
Sample concentration (nM)	500
Dissociation time (s)	600
Regeneration1	
Flow path	3,4
Flow rate (µl/min)	10
Regeneration contact time (s)	180
Regeneration buffer	10mM Glycine-HCl pH1.7
Stabilization time (s)	120
If …Then…Else	If after regeneration1 >20RU on Fc4
	Else exit cycle
Regeneration2	
Flow path	3,4
Flow rate (µl/min)	10
Regeneration contact time (s)	120
Regeneration buffer	10mM Glycine-HCl pH1.7
Stabilization time (s)	120

The above protocol was used to generate the 21-4 binding data set out in Table X. When the absolute values for RU were considered (after adjusting the measured RU value for the molecular weight of the ISV, protein or polypeptide according to the formula ([RU measured]/[MW of the protein] x 10^6), it was found that the Nanobodies and Nanobody constructs mentioned in Table X that had an added alanine residue and that showed >90% reduction in binding to both 21-4 as well as interference factors, generally provided RU values of between 30RU and 400RU (with the corresponding reference Nanobodies or polypeptides – as listed in Figure 9 – having RU values of more than 1000, usually more than 1500, and often more than 2000).

Based on this, it was considered that an (adjusted) RU value of less than 500 in this assay would be clearly indicative of an ISV(or a protein or polypeptide that comprises as least one IS, as described herein) that will (essentially) not be bound by interference factors in a manner that would interfere with an ADA assay.

The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced herein.

CLAIMS

1. Immunoglobulin single variable domain (ISV), which is either a Nanobody or an ISV that comprises a VH sequence (i.e. other than a Nanobody) or that is derived from a VH sequence, which ISV has a C-terminal end of the sequence $VTVSS(X)_n$, in which:

   - n = 1, 2 or 3 (and preferably 1 or 2) in which each X = Ala or Gly; or
   - n = 1, 2 or 3 (and preferably 1 or 2) in which each X = Ala; or
   - n = 1, 2 or 3 (and preferably 1 or 2) in which each X = Gly; or
   - n = 2 or 3 in which at least one X = Ala or Gly (with the remaining amino acid residue X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile); or
   - n = 2 or 3 in which all but one X = Ala or Gly (with the remaining amino acid residue X being independently chosen from any naturally occurring amino acid but preferably being independently chosen from Val, Leu and/or Ile);

   or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end.

2. Immunoglobulin single variable domain (ISV), protein or polypeptide according to claim 1, in which:

   - n = 1, 2 or 3 (and preferably 1 or 2) in which each X = Ala or Gly; or
   - n = 1, 2 or 3 (and preferably 1 or 2) in which each X = Ala; or
   - n = 1, 2 or 3 (and preferably 1 or 2) in which each X = Gly

3. Immunoglobulin single variable domain (ISV), protein or polypeptide according to claim 1, in which X is not cysteine.

4. Immunoglobulin single variable domain (ISV), which is either a Nanobody or an ISV that comprises a VH sequence or that is derived from a VH sequence, which ISV has a C-terminal end of the sequence VTVSS(X)n, in which n is 1 to 10, preferably 1 to 5, such as 1, 2, 3, 4 or 5 (and preferably 1 or 2, such as 1), and in which each X is an (preferably naturally occurring) amino acid residue that is independently chosen (and preferably

independently chosen from the group consisting of alanine (A), glycine (G), valine (V), leucine (L) or isoleucine (I), with the proviso that X is not cysteine; or a protein or polypeptide which contains such an ISV (and preferably such a Nanobody) at its C-terminal end.

5. Immunoglobulin single variable domain (ISV), protein or polypeptide according to any of claims 1-4, which in which said (C-terminal) ISV is a Nanobody.

6. Immunoglobulin single variable domain (ISV), protein or polypeptide according to any of claims 1-5 which has an RU value for binding by 21-4 of less than 500, as determined using Biacore according to the protocol set out in Example 9, and after adjusting the measured RU value for the molecular weight of the ISV, protein or polypeptide according to the formula ([RU measured]/[MW of the protein] x 10^6).

7. Method for predicting whether an ISV or protein or polypeptide comprising at least one ISV will give rise to protein interference in an immunoassay such as an ADA assay, said method comprising performing an immunoassay that at least comprises the steps of:
   (i) contacting said ISV or protein/polypeptide with an antibody that has been obtained from a human subject and that has been selected/isolated based on its ability to recognize and/or bind to the C-terminal end of said ISV; and
   (ii) determining whether said ISV, protein or polypeptide is bound by said antibody in said immunoassay.

8. Method according to claim 7, in which the ISV is either a Nanobody or an(other) ISV (i.e. other than a Nanobody) that is a VH domain or that comprises a VH domain.

9. Method according to claim 7 or 8, in which the ISV is a Nanobody.

10. Method according to any of claims 7 or 9, in which the protein or polypeptide has said ISV at its C-terminal end.

11. Method according to any of claims 7 to 10, in which the fact that the ISV, protein or polypeptide binds to said antibod in step (ii) means that the ISV, protein or polypeptide can give rise to (or has a high or increased risk of giving rise to) such protein interference.

12. Method according to any of claims 7 or 11, in which the antibody is a polyclonal antibody.

13. Method according to any of claims 7 to 12, in which the antibody is a polyclonal antibody that has been obtained, starting from a biological sample that has been obtained from a human subject and that is suitable as a starting material for obtaining polyclonal antibodies, by a method that comprises at least one step of (immuno)affinity chromatography in which affinity matrix is used that carries the ISV or protein or polypeptide comprising at least one ISV and/or in which the ISV or protein or polypeptide comprising at least one ISV is used as the affinity moiety or antigen, and optionally one or more further steps for isolating and/or purifying a polyclonal antibody from said sample (performed either before and/or after said affinity step).

14. Method according to claim 13, in which the ISV or protein or polypeptide comprising at least one ISV that is carried on the affinity matrix and/or that is used as the affinity moiety or antigen is an ISV or protein or polypeptide comprising at least one ISV that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33), or in which the ISV or protein or polypeptide comprising at least one ISV that is carried on the affinity matrix and/or that is used as the affinity moiety or antigen has at its C-terminal end an ISV or Nanobody that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33).

15. Method according to claim 13 or 14, in which that the ISV or protein or polypeptide comprising at least one ISV that is carried on the affinity matrix and/or that is used as the affinity moiety or antigen is an ISV or protein or polypeptide comprising at least one ISV, that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue on position 14; or in which the ISV or protein or polypeptide comprising at least one ISV that is carried on the affinity matrix and/or that is

used as the affinity moiety or antigen has at its C-terminal end is an ISV or protein or polypeptide comprising at least one ISV that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue on position 14.

16. Method according to any of claims 13 to 15, in which that the ISV or Nanobody that is carried on the affinity matrix and/or that is used as the affinity moiety or antigen is a sequence-optimized and/or humanized Nanobody (such as a sequence-optimized and/or humanized VHH or a camelized VH, such as a camelized human VH); or in which the ISV-based drug or Nanobody-based drug that is carried on the affinity matrix and/or that is used as the affinity moiety or antigen has at its C-terminal end an ISV or Nanobody that is a sequence-optimized and/or humanized Nanobody (such as a sequence-optimized and/or humanized VHH or a camelized VH, such as a camelized human VH).

17. Method according to any of claims 15 or 16, in which that the ISV or Nanobody that is carried on the affinity matrix and/or that is used as the affinity moiety or antigen is a sequence-optimized and/or humanized Nanobody that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue on position 14 which has been introduced as part of the humanization and/or sequence optimization of the corresponding naturally occurring VHH; or in which the ISV-based drug or Nanobody-based drug that is carried on the affinity matrix and/or that is used as the affinity moiety or antigen has at its C-terminal end a sequence-optimized and/or humanized Nanobody that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue on position 14 which has been introduced as part of the humanization and/or sequence optimization of the corresponding naturally occurring VHH.

18. Method according to any of claims 7 to 11, in which the antibody is a monoclonal antibody.

19. Method according to any of claims 7 to 11 or 18, in which the antibody is a polyclonal antibody that has been obtained, starting from a biological sample that has been obtained from a human subject and that is suitable as a starting material for obtaining monoclonal,

by a method that comprises at least one screening or selection step in which an ISV, Nanobody, ISV-based drug or Nanobody-based drug is used for screening and selecting a monoclonal antibody that binds to said ISV, Nanobody, ISV-based drug or Nanobody-based drug (and in particular to the C-terminal end of the same), and optionally one or more further steps for isolating and/or purifying a monoclonal antibody from said sample (performed either before and/or after said screening and/or selection step(s)).

20. Method according to claim 19, in which the ISV or Nanobody that is used in the screening or selection step ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33), or in which the ISV-based drug or Nanobody-based drug that is used in the screening or selection step has at its C-terminal end an ISV or Nanobody that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33).

21. Method according to claim 19 or 20, in which the ISV or Nanobody that is used in the screening or selection step ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue at position 14, or in which the ISV-based drug or Nanobody-based drug that is used in the screening or selection step has at its C-terminal end an ISV or Nanobody that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue at position 14.

22. Method according to claim 19, 20 or 21, in which the ISV or Nanobody that is used in the screening or selection step is a sequence-optimized and/or humanized Nanobody (such as a sequence-optimized and/or humanized VHH or a camelized VH, such as a camelized human VH); or in which the ISV-based drug or Nanobody-based drug that is used in the screening or selection step has at its C-terminal end an ISV or Nanobody that is a sequence-optimized and/or humanized Nanobody (such as a sequence-optimized and/or humanized VHH or a camelized VH, such as a camelized human VH).

23. Method according to any of claims 21 to 22, in which the ISV or Nanobody that is used in the screening or selection step is a sequence-optimized and/or humanized Nanobody that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and

that has a proline residue on position 14 which has been introduced as part of the humanization and/or sequence optimization of the corresponding naturally occurring VHH; or in which the ISV-based drug or Nanobody-based drug that is used in the screening or selection step has at its C-terminal end a sequence-optimized and/or humanized Nanobody that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue on position 14 which has been introduced as part of the humanization and/or sequence optimization of the corresponding naturally occurring VHH.

24. Method that can be used to predict whether an ISV or protein or polypeptide comprising at least one ISV will give rise to (or has high or increased tendency to give rise to) protein interference in an immunoassay (and/or to predict whether said ISV or protein or polypeptide comprising at least one ISV will be bound by interference factor(s) present in the blood or serum of a human being), said method comprising performing an immunoassay that at least comprises the steps of:

(i) contacting said ISV or Nanobody (or ISV-based or Nanobody-based drug) with the monoclonal antibody 21-4 (i.e. used as the "*analytical antibody*"); and

(ii) determining whether said ISV or Nanobody (or ISV-based or Nanobody-based drug) is bound by the monoclonal antibody 21-4 in said immunoassay.

25. Method according to claim 24, in which the ISV is either a Nanobody or an(other) ISV (i.e. other than a Nanobody) that is a VH domain or that comprises a VH domain.

26. Method according to claim 24 or 25, in which the ISV is a Nanobody.

27. Method according to any of claims 24 to 27, in which the protein or polypeptide has said ISV at its C-terminal end.

28. Method according to any of claims 24 to 27, that is performed according to the protocol said out in Example 9.

29. Pharmaceutical composition that comprises an ISV, protein or polypeptide according to any of claims 1-6, and at least one suitable carrier, diluent or excipient.

30. Pharmaceutical composition according to claim 29, in which:

- said composition, ISV, protein or polypeptide is intended for treatment of a chronic disease in a human being, and/or
- said ISV, protein, polypeptide is intended to be present in the circulation of the subject (i.e. at pharmacologically active levels) to which it is administered (i.e. at a therapeutically active dose) for at least a period of one week, preferably at least two weeks, such as at least a months; and/or
- said ISV, protein, polypeptide is such that it has a half-life (preferably expressed as t1/2-beta) in a human subject of at least 3 days, such as at least one week, and up to 10 days or more; and/or such
- said ISV, protein, polypeptide or pharmaceutical composition is intended to be administered to a human being as two or more doses that are administered over a period of at least 3 days, such as at least one week, for example at least two weeks or at least one month, or even longer (i.e. at least 3 months, at least 6 months or at least one year), or even chronically administered.

31. ISV, protein or polypeptide according to any of claims 1-6 for use in therapy of a disease in a human being.

32. ISV, protein or polypeptide according to any of claims 1-6 and/or 31, in which:

- said ISV, protein or polypeptide is intended for treatment of a chronic disease in a human being, and/or
- said ISV, protein, polypeptide is intended to be present in the circulation of the subject (i.e. at pharmacologically active levels) to which it is administered (i.e. at a therapeutically active dose) for at least a period of one week, preferably at least two weeks, such as at least a months; and/or
- said ISV, protein, polypeptide is such that it has a half-life (preferably expressed as t1/2-beta) in a human subject of at least 3 days, such as at least one week, and up to 10 days or more; and/or such

- said ISV, protein or polypeptide is intended to be administered to a human being as two or more doses that are administered over a period of at least 3 days, such as at least one week, for example at least two weeks or at least one month, or even longer (i.e. at least 3 months, at least 6 months or at least one year), or even chronically administered.

33. Use of an ISV or a protein or polypeptide according to any of claims 1-6, 31 or 32 in the preparation of a pharmaceutical composition, and in particular in the preparation of a pharmaceutical composition according to claim 29 or 30.

34. Use according to claim 33, in which

- said ISV, protein or polypeptide is intended for treatment of a chronic disease in a human being, and/or
- said ISV, protein, polypeptide is intended to be present in the circulation of the subject (i.e. at pharmacologically active levels) to which it is administered (i.e. at a therapeutically active dose) for at least a period of one week, preferably at least two weeks, such as at least a months; and/or
- said ISV, protein, polypeptide is such that it has a half-life (preferably expressed as t1/2-beta) in a human subject of at least 3 days, such as at least one week, and up to 10 days or more; and/or such
- said ISV, protein or polypeptide is intended to be administered to a human being as two or more doses that are administered over a period of at least 3 days, such as at least one week, for example at least two weeks or at least one month, or even longer (i.e. at least 3 months, at least 6 months or at least one year), or even chronically administered.

35. Method of treatment which comprises administering to a human subject (e.g to a patient in need of such treatment) an ISV or a protein or polypeptide according to one of claims 1-6, 31 or 32 or a pharmaceutical composition according to claim 29 or 30.

36. Method of treatment according to claim 35, in which:

- said pharmaceutical composition, ISV, protein or polypeptide is intended for treatment of a chronic disease in a human being, and/or

- said ISV, protein, polypeptide is intended to be present in the circulation of the subject (i.e. at pharmacologically active levels) to which it is administered (i.e. at a therapeutically active dose) for at least a period of one week, preferably at least two weeks, such as at least a months; and/or
- said ISV, protein, polypeptide is such that it has a half-life (preferably expressed as t1/2-beta) in a human subject of at least 3 days, such as at least one week, and up to 10 days or more; and/or such
- said pharmaceutical composition, ISV, protein or polypeptide is intended to be administered to a human being as two or more doses that are administered over a period of at least 3 days, such as at least one week, for example at least two weeks or at least one month, or even longer (i.e. at least 3 months, at least 6 months or at least one year), or even chronically administered.

ABSTRACT

This invention provides, and in certain specific but non-limiting aspects relates to: assays that can be used to predict whether a given ISV will be subject to protein interference as described herein and/or give rise to an (aspecific) signal in such an assay (such as for example in an ADA immunoassay). Such predictive assays could for example be used to test whether a given ISV could have a tendency to give rise to such protein interference and/or such a signal; to select ISV's that are not or less prone to such protein interference or to giving such a signal; as an assay or test that can be used to test whether certain modification(s) to an ISV will (fully or partially) reduce its tendency to give rise to such interference or such a signal; and/or as an assay or test that can be used to guide modification or improvement of an ISV so as to reduce its tendency to give rise to such protein interference or signal; --methods for modifying and/or improving ISV's to as to remove or reduce their tendency to give rise to such protein interference or such a signal; -- modifications that can be introduced into an ISV that remove or reduce its tendency to give rise to such protein interference or such a signal; ISV's that have been specifically selected (for example, using the assay(s) described herein) to have no or low(er)/reduced tendency to give rise to such protein interference or such a signal; modified and/or improved ISV's that have no or a low(er)/reduced tendency to give rise to such protein interference or such a signal.

Figure 1A

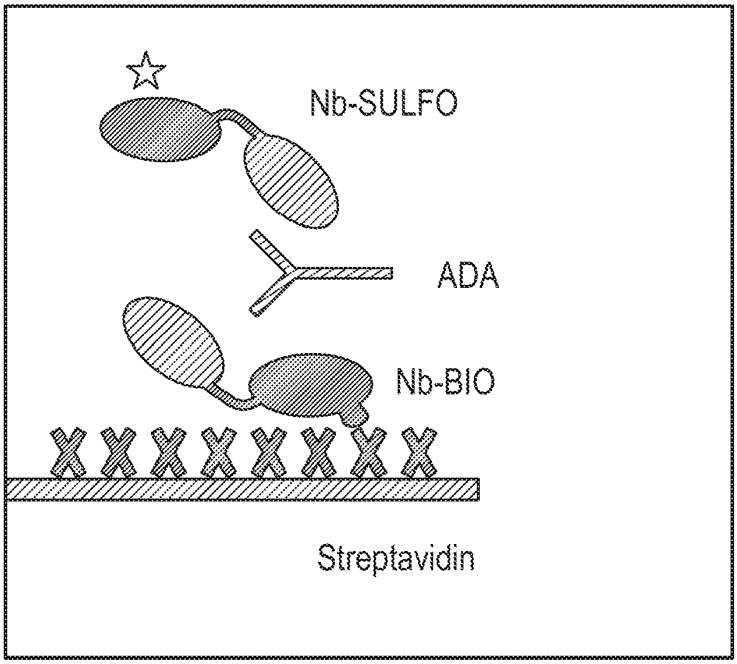


Figure 1B

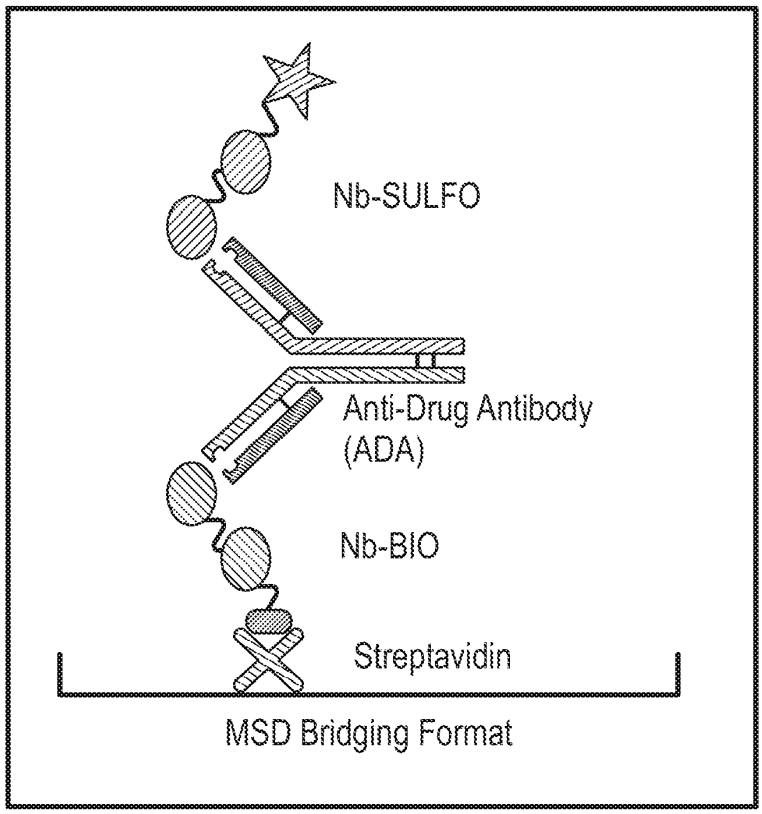

Nb-SULFO
Anti-Drug Antibody
(ADA)
Nb-BIO
Streptavidin
MSD Bridging Format

Figure 1C

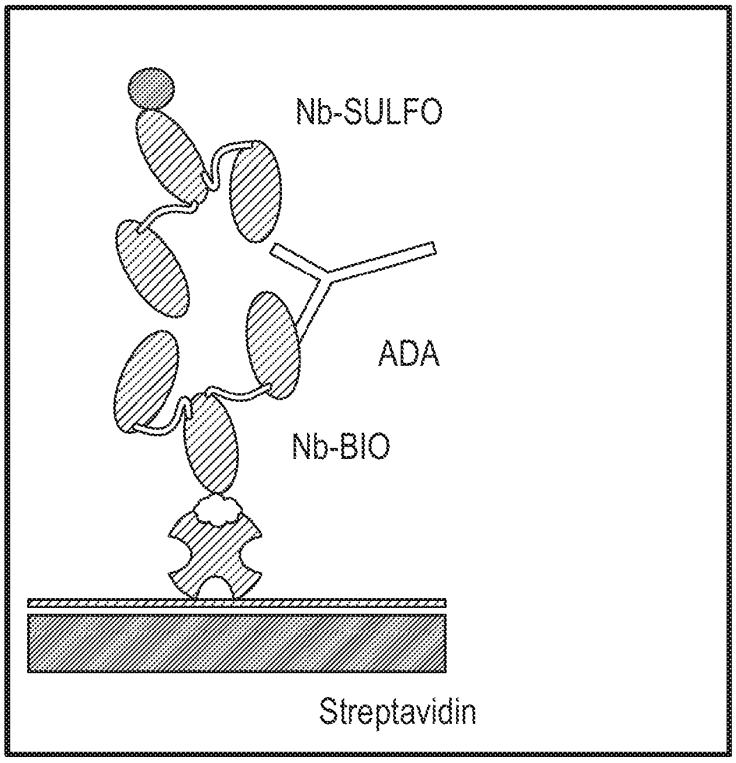


Figure 2

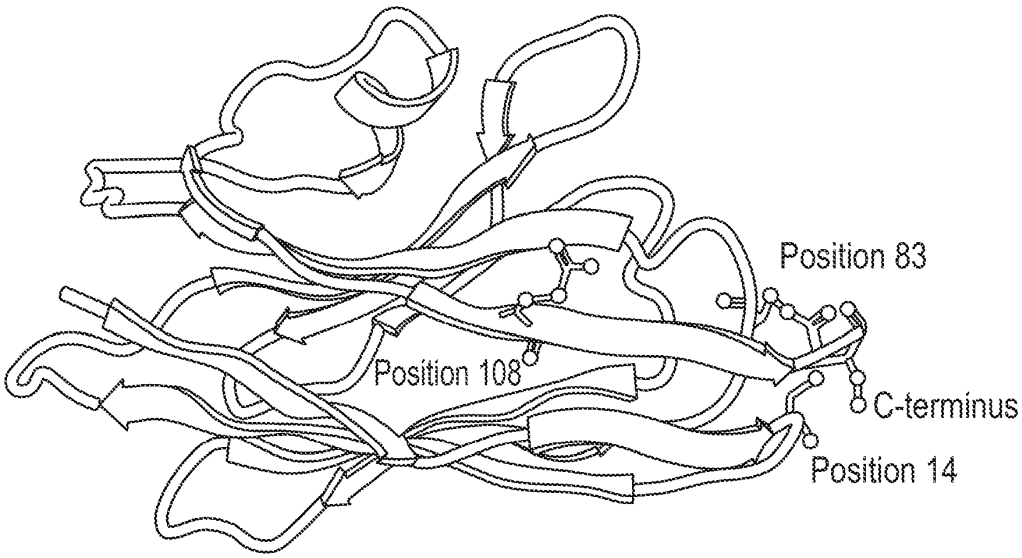
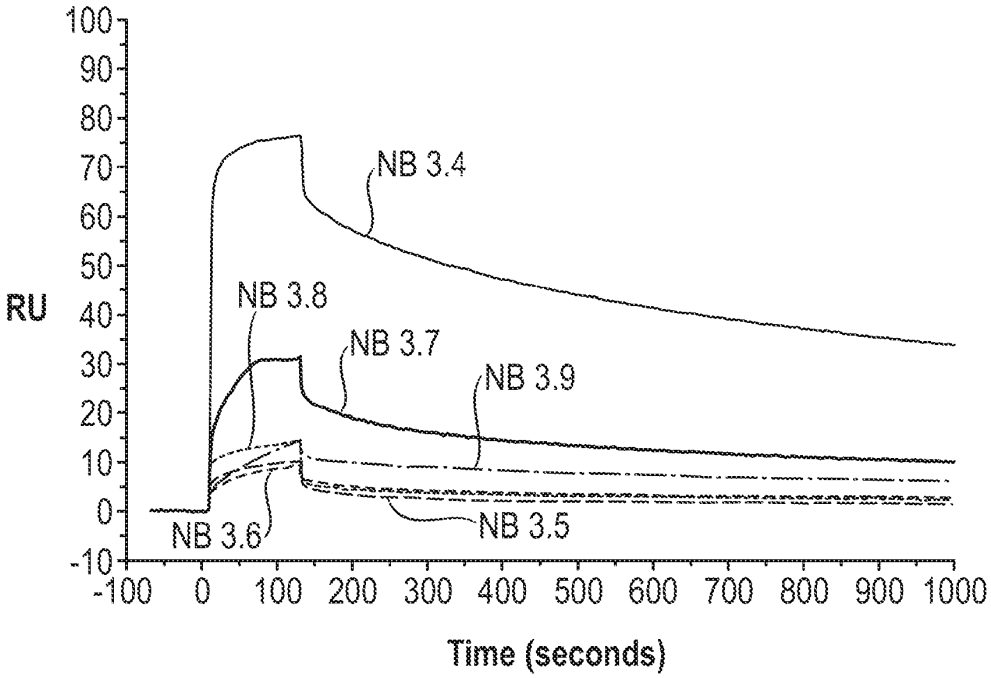


Figure 3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Time (seconds)
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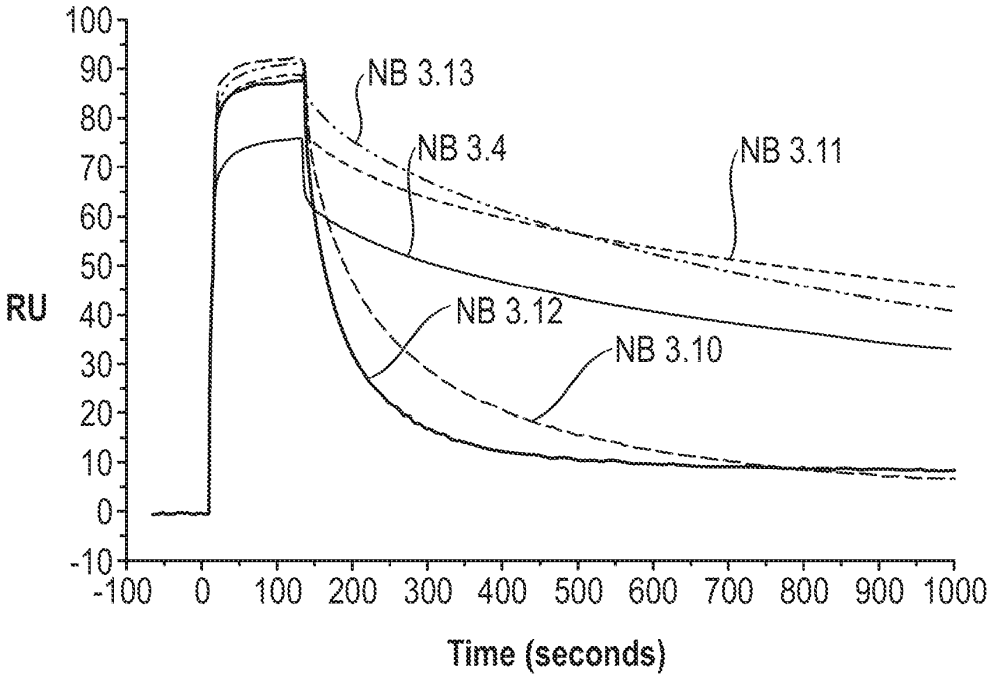
NB 3.8
NB 3.7
NB 3.9
NB 3.6
NB 3.5
100
90
80
70
60
50
40
30
20
10
0
-10
-100
0
100
200
300
400
500
600
700
800
900
1000

Figure 4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NB 3.4
NB 3.11
NB 3.12
NB 3.10
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70
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40
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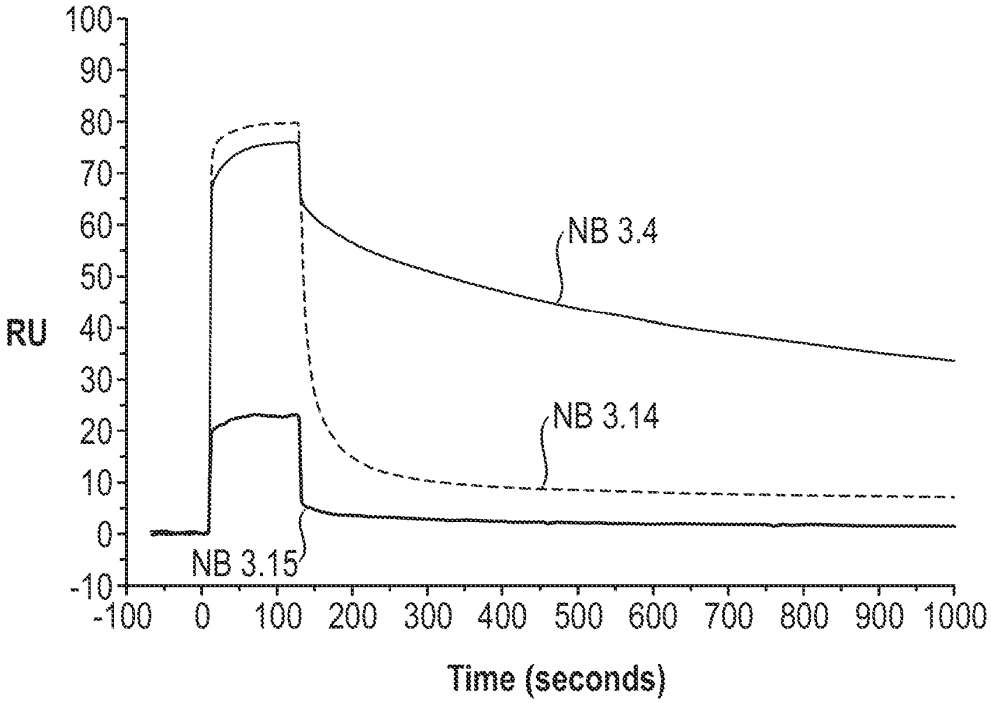
0
-10
-100
0
100
200
300
400
500
600
700
800
900
1000

Figure 5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NB 3.14
NB 3.15
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-10
-100
0
100
200
300
400
500
600
700
800
900
1000

Figure 6

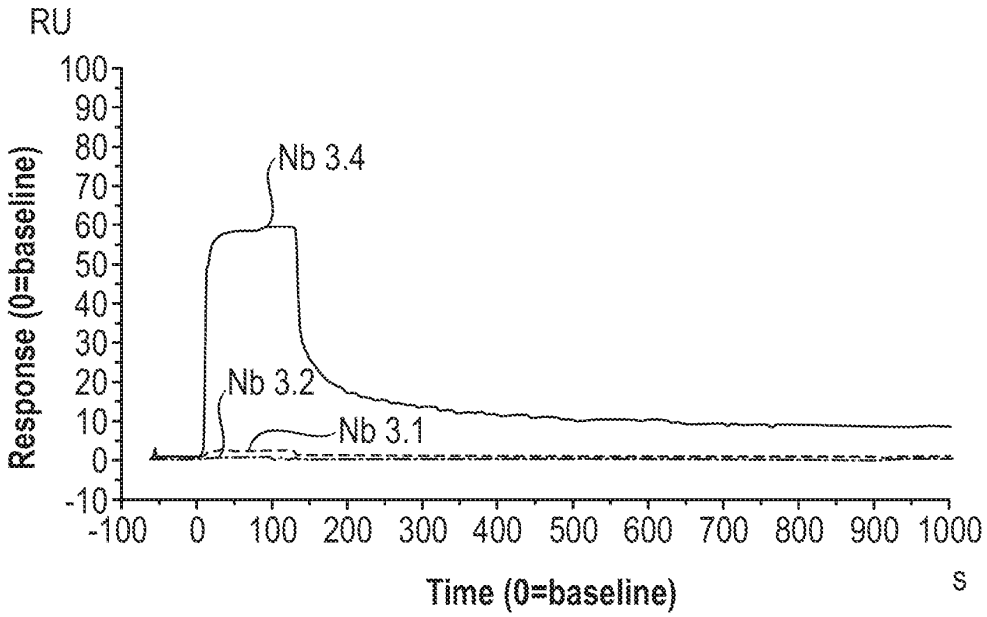
RU
100
90
80
70
60
50
40
30
20
10
0
-10
Response (0=baseline)
Nb 3.4
Nb 3.2
Nb 3.1
-100
0
100
200
300
400
500
600
700
800
900
1000
Time (0=baseline)
s

Figure 7

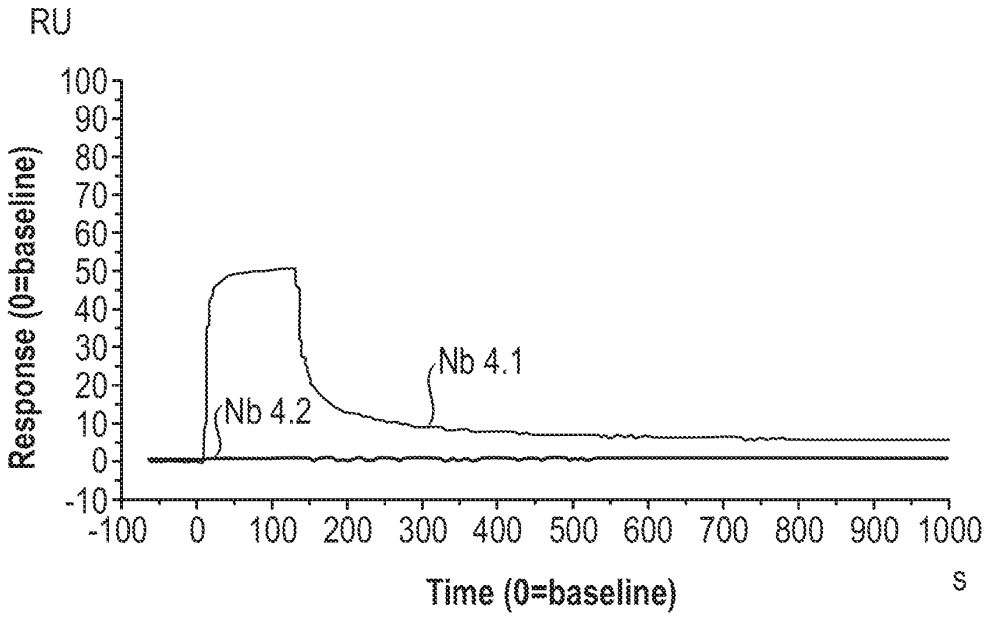
RU
100
90
80
70
60
50
40
30
20
10
0
-10
Response (0=baseline)
Nb 4.1
Nb 4.2
-100
0
100
200
300
400
500
600
700
800
900
1000
Time (0=baseline)
s

Figure 8

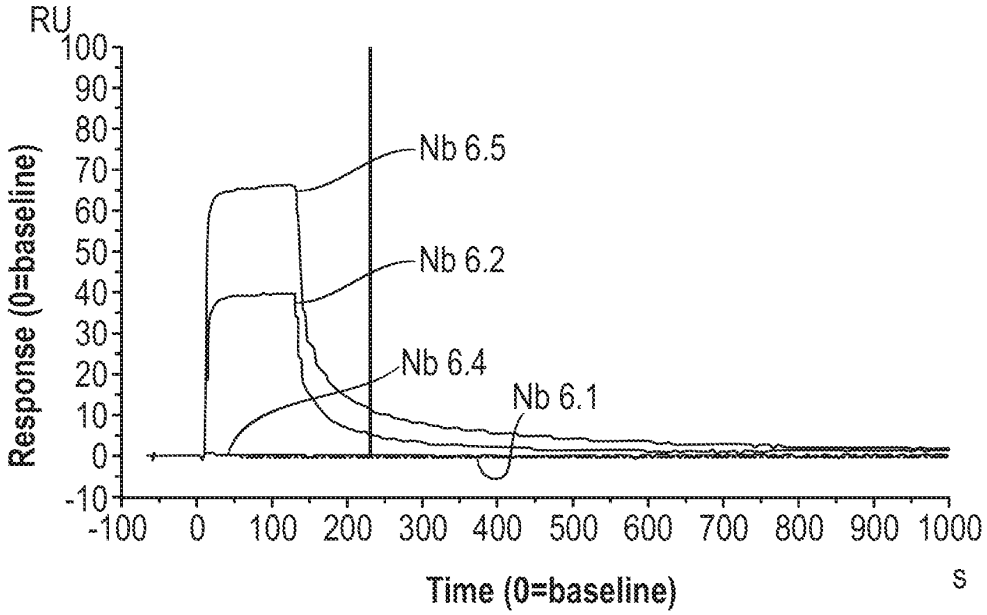
RU
100
90
80
70
60
50
40
30
20
10
0
-10
Response (0=baseline)
Nb 6.5
Nb 6.2
Nb 6.4
Nb 6.1
-100
0
100
200
300
400
500
600
700
800
900
1000
Time (0=baseline)
s

Figure 9

SEQ ID NO	N-terminal amino acid(s)	Mutations in the C-terminal region	Sequence	Reference sequence
37	A	none	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSA	SEQ ID NO: 37 without the added C-terminal amino acid residues
38	A	none	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSGIKSSGDSTRYAGSVKGRFTISRDNAKN TLYLQMNSLRPEDTAVYYCAKSRVSRTGLYTYDNRGQG TLVTVSSGGGGSGGGGSGGGGSEVQLVESGGGL VQPGGSLRLSCAASGRTFNNYAMGWFRQAPGKEREFVA AITRSGVRSGVSAIYGDSVKDRFTISRDNAKNTLYLQMN SLRPEDTAVYYCAASAIGSGALRRFEYDYSGQGTLVTVS SA	SEQ ID NO: 38 without the added C-terminal amino acid residues
39	A	none	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYPMGWFR QAPGKGREFVSSITGSGGSTYYADSVKGRFTISRDNAKN TLYLQMNSLRPEDTAVYYCAAYIRPDTYLSRDYRKYDY WGQGTLVTVSSGGGGSGGGGSEVQLVESGGGLVQPGNSL RLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSD TLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYY CTIGGSLSRSSQGTLVTVSSGGGGSGGGGSEVQLVESGGG LVQPGGSLRLSCAASGFTFSSYPMGWFRQAPGKGREFVS SITGSGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLRP EDTAVYYCAAYIRPDTYLSRDYRKYDYWGQGTLVTVSS A	SEQ ID NO: 39 without the added C-terminal amino acid residues

Figure 9 (continued)

40	A	none	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWV RQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAK NTLYLQMNSLRPEDTAVYYCARSPSGFNRGQGTLVTVS SGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTF SSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGR FTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQ GTLVTVSSGGGSGGGSEVQLVESGGGLVQPGGSLRLSC AASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAR SPSGFNRGQGTLVTVSSA	SEQ ID NO: 40 without the added C-terminal amino acid residues
41	A	none	EVQLVESGGGLVQPGGSLRLSCAASGSVFKINVMAWYR QAPGKGRELVAGIISGGSTSYADSVKGRFTISRDNAKNTL YLQMNSLRPEDTAVYYCAFITTESDYDLGRRYWGQGTL VTVSSGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAA SGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADS VKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSL SRSSQGTLVTVSSA	SEQ ID NO: 41 without the added C-terminal amino acid residues
42	A	none	EVQLVESGGGLVQPGGSLRLSCAASGRTFSYNPMGWFR QAPGKGRELVAAISRTGGSTYYPDSVEGRFTISRDNAKR MVYLQMNSLRAEDTAVYYCAAAGVRAEDGRVRTLPSE YTFWGQGTQVTVSSAAAEVQLVESGGGLVQPGGSLRLS CAASGRTFSYNPMGWFRQAPGKGRELVAAISRTGGSTY YPDSVEGRFTISRDNAKRMVYLQMNSLRAEDTAVYYCA AAGVRAEDGRVRTLPSEYTFWGQGTQVTVSSA	SEQ ID NO: 42 without the added C-terminal amino acid residues

Figure 9 (continued)

43	A	none	DVQLVESGGGLVQPGGSLRLSCAASGFILDYYAIGWFRQ APGKEREGVLCIDASDDITYYADSVKGRFTISRDNSKNT VYLQMNSLRPEDTAVYYCATPIGLSSSCLLEYDYDYWG QGTLVTVSSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLS CAASGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLY ADSVKGRFTISRDNSKNTLYLQMNSLRPEDTAVYYCTIG GSLSRSSQGTLVTVSSA	SEQ ID NO: 43 without the added C-terminal amino acid residues
44	A	none	DVQLVESGGGLVQPGGSLRLSCAASRSIGRLDRMGWYR HRPGEPRELVATITTGGSSINYGDSVKGRFTISIDNSKNTV YLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQGTLVTV SSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFT FSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKG RFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSS QGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRL SCAASRSIGRLDRMGWYRHRPGEPRELVATITTGGSSINY GDSVKGRFTISIDNSKNTVYLQMNSLRPEDTAVYYCNFN KYVTSRDTWGQGTLVTVSSA	SEQ ID NO: 44 without the added C-terminal amino acid residues
45	none	P14A, P41T, S62F, S74A, S82bN, R83K, L108Q	EVQLVESGGGLVQAGGSLRLSCAASRSIGRLDRMGWYR HRTGEPRELVATITTGGSSINYGDFVKGRFTISIDNAKNTV YLQMNNLKPEDTAVYYCNFNKYVTSRDTWGQGTQVTV SS	SEQ ID NO: 45 without the added C-terminal amino acid residues and without the mentioned mutations in the C-terminal region
46	AAEQKLIS EEDLNGA AHHHHHH	A14P, T41P, F62S, A74S, N82bS, K83R, Q108L	EVQLVESGGGLVQAGGSLRLSCAASRSIGRLDRMGWYR HRTGEPRELVATITTGGSSINYGDFVKGRFTISIDNAKNTV YLQMNNLKPEDTAVYYCNFNKYVTSRDTWGQGTQVTV SSAAAHHHHHHHGAAEQKLISEEDLNGAA	SEQ ID NO: 46 without the added C-terminal amino acid residues and without the mentioned mutations in the C-terminal region

Figure 9 (continued)

47	GGGGSGG GSRDWDF DVFGGGT PVGG	none	EVQLVESGGGLVQPGGSLRLSCAASGSVFKINVMAWYR QAPGKGRELVAGIISGGSTSYADSVKGRFTISRDNAKNTL YLQMNSLRPEDTAVYYCAFITTESDYDLGRRYWGQGTL VTVSSGGGGSGGGSRDWDFDVFGGGTPVGG	SEQ ID NO: 47 without the added C-terminal amino acid residues
48	AAEQKLIS EEDLNGA AHHHHHH	none	EVQLVESGGGLVQPGGSLRLSCIASGLPFSTKSMGWFRQ APGKEREFVARISPGGTSRYYGDFVKGRFAISRDNAKNT TWLQMNSLKAEDTAVYYCASGERSTYIGSNYYRTNEYD YWGTGTQVTVSSAAAEQKLISEEDLNGAAHHHHHH	SEQ ID NO: 48 without the added C-terminal amino acid residues
49	AAEQKLIS EEDLNGA AHHHHHH	V5L, I23A, E44G, A49S, A68T, A74S, T78L, W79Y, K83R, T110Q, Q108L	EVQLLESGGGLVQPGGSLRLSCAASGLPFSTKSMGWFR QAPGKGREFVSRISPGGTSRYYGDFVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYCASGERSTYIGSNYYRTNEY DYWGQGTLVTVSSAAAEQKLISEEDLNGAAHHHHHH	SEQ ID NO: 49 without the added C-terminal amino acid residues and without the mentioned mutations in the C-terminal region
50	none	L11S	HHHHHHHEVQLVESGGGSVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSS	SEQ ID NO: 50 without the mentioned mutations in the C-terminal region
51	none	T110Q	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV QVSS	SEQ ID NO: 51 without the mentioned mutations in the C-terminal region

Figure 9 (continued)

52	none	S112G	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVGS	SEQ ID NO: 52 without the mentioned mutations in the C-terminal region
53	none	S113G	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSG	SEQ ID NO: 53 without the mentioned mutations in the C-terminal region
54	none	L11S, T110Q, S112G, S113G	HHHHHHHEVQLVESGGGSVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV QVGG	SEQ ID NO: 54 without the mentioned mutations in the C-terminal region
55	A	none	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSA	SEQ ID NO: 55 without the added C-terminal amino acid residues
56	G	S113G	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSGG	SEQ ID NO:56 without the added C-terminal amino acid residues and without the mentioned mutations in the C-terminal region
57	AS	none	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSAS	SEQ ID NO:57 without the added C-terminal amino acid residues

Figure 9 (continued)

58	AST	none	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSAST	SEQ ID NO:58 without the added C-terminal amino acid residues
59	ASTK	none	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSASTK	SEQ ID NO:59 without the added C-terminal amino acid residues
60	ASP	none	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSASP	SEQ ID NO:60 without the added C-terminal amino acid residues
61	AP	none	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSAP	SEQ ID NO:61 without the added C-terminal amino acid residues
62	APT	none	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSAPT	SEQ ID NO:62 without the added C-terminal amino acid residues
63	W	none	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSW	SEQ ID NO:63 without the added C-terminal amino acid residues
64	L	none	HHHHHHHEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFG MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISR DNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSSL	SEQ ID NO:64 without the added C-terminal amino acid residues

Figure 9 (continued)

65	none	P14A	HHHHHHEVQLVESGGGLVQAGGSLRLSCAASRSIGRLD RMGWYRHRPGEPRELVATITTGGSSINYGDSVKGRFTISID NSKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQ GTLVTVSS	SEQ ID NO:65 without the mentioned mutations in the C-terminal region
66	none	L11S	HHHHHHHEVQLVESGGGSVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITTGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVSS	SEQ ID NO:66 without the mentioned mutations in the C-terminal region
67	none	R83K	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITTGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLKPEDTAVYYCNFNKYVTSRDTWGQG TLVTVSS	SEQ ID NO:67 without the mentioned mutations in the C-terminal region
68	none	P14A, L108Q	HHHHHHHEVQLVESGGGLVQAGGSLRLSCAASRSIGRLD RMGWYRHRPGEPRELVATITTGGSSINYGDSVKGRFTISID NSKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQ GTQVTVSS	SEQ ID NO:68 without the mentioned mutations in the C-terminal region
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71	none	S113G	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITTGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVSG	SEQ ID NO:71 without the mentioned mutations in the C-terminal region

Figure 9 (continued)

72	none	S112G, S113G	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVGG	SEQ ID NO:72 without the mentioned mutations in the C-terminal region
73	G	S112G, S113G	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVGGG	SEQ ID NO:73 without the added C-terminal amino acid residues and without the mentioned mutations in the C-terminal region
74	G	none	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVSSG	SEQ ID NO:74 without the added C-terminal amino acid residues
75	AA	none	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVSSAA	SEQ ID NO:75 without the added C-terminal amino acid residues
76	GGG	none	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVSSGGG	SEQ ID NO:76 without the added C-terminal amino acid residues
77	A	none	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVSSA	SEQ ID NO:77 without the added C-terminal amino acid residues

Figure 9 (continued)

78	none	Q13R	HHHHHHHEVQLVESGGGLVRPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVSS	SEQ ID NO:78 without the mentioned mutations in the C-terminal region
79	GG	none	HHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVSSGG	SEQ ID NO:79 without the added C-terminal amino acid residues
80	none	T110Q, S112G, S113G	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVQVGG	SEQ ID NO:80 without the mentioned mutations in the C-terminal region
81	none	L11V	HHHHHHHEVQLVESGGGVVQPGGSLRLSCAASRSIGRLD RMGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISID NSKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQ GTLVTVSS	SEQ ID NO:81 without the mentioned mutations in the C-terminal region
82	none	P84A	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRAEDTAVYYCNFNKYVTSRDTWGQG TLVTVSS	SEQ ID NO:82 without the mentioned mutations in the C-terminal region
83	none	T87A	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDAAVYYCNFNKYVTSRDTWGQG TLVTVSS	SEQ ID NO:83 without the mentioned mutations in the C-terminal region
84	none	S112G	HHHHHHHEVQLVESGGGLVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVGS	SEQ ID NO:84 without the mentioned mutations in the C-terminal region

Figure 9 (continued)

85	none	L11S, T110Q, S112G, S113G	HHHHHHHEVQLVESGGGSVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVQVGG	SEQ ID NO:85 without the mentioned mutations in the C-terminal region
86	none	L11S, T110Q	HHHHHHHEVQLVESGGGSVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVQVSS	SEQ ID NO:86 without the mentioned mutations in the C-terminal region
87	none	L11S, S112G, S113G	HHHHHHHEVQLVESGGGSVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVTVGG	SEQ ID NO:87 without the mentioned mutations in the C-terminal region
88	A	L11S, T110Q	HHHHHHHEVQLVESGGGSVQPGGSLRLSCAASRSIGRLDR MGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISIDN SKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQG TLVQVSSA	SEQ ID NO:88 without the added C-terminal amino acid residues and without the mentioned mutations in the C-terminal region
89	none	L11S, P14A, T110Q, S112G, S113G	HHHHHHHEVQLVESGGGSVQAGGSLRLSCAASRSIGRLD RMGWYRHRPGEPRELVATITGGSSINYGDSVKGRFTISID NSKNTVYLQMNSLRPEDTAVYYCNFNKYVTSRDTWGQ GTLVQVGG	SEQ ID NO:89 without the mentioned mutations in the C-terminal region

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If you need help call the Patent Electronic Business Center at (866)
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Arg Met Gly Trp Tyr Arg His Arg Thr Gly Glu Pro Arg Glu Leu Val
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Ala Thr Ile Thr Gly Gly Ser Ser Ile Asn Tyr Gly Asp Phe Val Lys
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Gly Arg Phe Thr Ile Ser Ile Asp Asn Ala Lys Asn Thr Val Tyr Leu
65                  70                  75                  80


Gln Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn
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Val Thr Val Ser Ser
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Gly Arg Phe Thr Ile Ser Ile Asp Asn Ala Lys Asn Thr Val Tyr Leu
65                  70                  75                  80

Gln Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn
                85                  90                  95

Phe Asn Lys Tyr Val Thr Ser Arg Asp Thr Trp Gly Gln Gly Thr Gln
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Arg Met Gly Trp Tyr Arg His Arg Pro Gly Glu Pro Arg Glu Leu Val
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Gly Arg Phe Thr Ile Ser Ile Asp Asn Ser Lys Asn Thr Val Tyr Leu
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Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn
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Ser Ile Gly Arg Leu Asp Arg Met Gly Trp Tyr Arg His Arg Pro Gly
        35                  40                  45

Glu Pro Arg Glu Leu Val Ala Thr Ile Thr Gly Gly Ser Ser Ile Asn
    50                  55                  60

Tyr Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ile Asp Asn Ser
65                  70                  75                  80

Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
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Ala Val Tyr Tyr Cys Asn Phe Asn Lys Tyr Val Thr Ser Arg Asp Thr
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Glu Pro Arg Glu Leu Val Ala Thr Ile Thr Gly Gly Ser Ser Ile Asn
    50                  55                  60


Tyr Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ile Asp Asn Ser
65                  70                  75                  80


Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
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        35                  40                  45

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Tyr Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ile Asp Asn Ser
65                  70                  75                  80

Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
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Tyr Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ile Asp Asn Ser
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Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
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Ser Ile Gly Arg Leu Asp Arg Met Gly Trp Tyr Arg His Arg Pro Gly
        35                  40                  45

Glu Pro Arg Glu Leu Val Ala Thr Ile Thr Gly Gly Ser Ser Ile Asn
    50                  55                  60

Tyr Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ile Asp Asn Ser
65                  70                  75                  80

Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
                85                  90                  95

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Glu Pro Arg Glu Leu Val Ala Thr Ile Thr Gly Gly Ser Ser Ile Asn
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Tyr Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ile Asp Asn Ser
65 70 75 80

Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
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Glu Pro Arg Glu Leu Val Ala Thr Ile Thr Gly Gly Ser Ser Ile Asn
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Tyr Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ile Asp Asn Ser
65                  70                  75                  80

Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
                85                  90                  95

Ala Val Tyr Tyr Cys Asn Phe Asn Lys Tyr Val Thr Ser Arg Asp Thr
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser
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PTO/AIA/15 (10-17)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	A0848.70142US12
First Named Inventor	Judith Baumeister
Title	TECHNIQUES FOR PREDICTING, DETECTING AND REDUCING ASPECIFIC PROTEIN INTERFERENCE IN ASSAYS INVOLVING IMMUNOGLOBULIN SINGLE VARIABLE DOMAINS
Priority Mail Express® Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [X] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [ ] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [X] **Specification** [*Total Pages* 96] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. [X] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 20]
6. **Inventor's Oath or Declaration** [*Total Pages* 18] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. [ ] Newly executed (original or copy)
   b. [X] A copy from a prior application (37 CFR 1.63(d))
7. [X] **Application Data Sheet** * *See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. [ ] Computer Readable Form (CRF)
   b. [ ] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] Paper
   c. [ ] Statements verifying identity of above copies

ADDRESS TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**

ACCOMPANYING APPLICATION PAPERS

10. [ ] **Assignment Papers** (cover sheet & document(s))
    Name of Assignee
11. [ ] **37 CFR 3.73(c) Statement** (*when there is an assignee*) [ ] **Power of Attorney**
12. [ ] **English Translation Document** (*if applicable*)
13. [ ] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
14. [ ] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [ ] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [X] **Other:** Sequence Listing (Text File)

***Note:** (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

[X] The address associated with Customer Number: 159933 **OR** [ ] Correspondence address below

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Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Curtis R. Powell/	Date	August 23, 2021
Name (Print/Type)	Curtis R. Powell	Registration No. (Attorney/Agent)	73,995

Application Data Sheet

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Postal or Zip Code of mailing address::	9052

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Country of mailing address::	Belgium
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City of mailing address::	Zwijnaarde
Country of mailing address::	Belgium
Postal or Zip Code of mailing address::	9052

Correspondence Information

Correspondence Customer Number::	159933

Application Information

Application Type::	Nonprovisional
Subject Matter::	Utility
CD-ROM or CD-R?::	None
Sequence submission?::	Yes
Computer Readable Form (CRF)?::	Text File
Title::	TECHNIQUES FOR PREDICTING, DETECTING AND REDUCING ASPECIFIC PROTEIN INTERFERENCE IN ASSAYS INVOLVING IMMUNOGLOBULIN SINGLE VARIABLE DOMAINS
Attorney Docket Number::	A0848.70142US12
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets::	20
Small Entity?::	No
Petition included?::	No
Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2::	No
This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013::	No

Representative Information

Representative Customer Number:: 159933

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::	Prior Appl Status::
This Application	Continuation of	14/128681	03/04/14	Pending
14/128681	National Stage of	PCT/EP2012/062251	06/25/12	
PCT/EP2012/062251	An application claiming the benefit under 35 USC 119(e)	61/500360	06/23/11	Expired
PCT/EP2012/062251	An application claiming the benefit under 35 USC 119(e)	61/500464	06/23/11	Expired
PCT/EP2012/062251	An application claiming the benefit under 35 USC 119(e)	61/541368	09/30/11	Expired
PCT/EP2012/062251	Continuation-in-part of	PCT/EP2011/067132	09/30/11	
PCT/EP2012/062251	Claims benefit of provisional	13/435567	03/30/12	Granted
PCT/EP2012/062251	Claims benefit of provisional	PCT/EP2012/061304	06/14/12	

Foreign Priority Information

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Country of mailing address::	Belgium
Postal or Zip Code of mailing address::	9052

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Assignee Number::	1
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Street of mailing address::	Technologiepark 21
City of mailing address::	Zwijnaarde
Country of mailing address::	Belgium
Postal or Zip Code of mailing address::	9052

Authorization or Opt-Out of Authorization to Permit Access

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Curtis R. Powell/	Date (YYYY-MM-DD)	2021-08-23
Name	Curtis R. Powell	Registration Number	73,995

LISTING OF THE CLAIMS

No claims have been canceled, amended or added. Nonetheless, Applicants present below a full listing of the pending claims for the Examiner's reference.

1.-50. (Canceled)

51. (Previously presented) A fusion protein comprising at least two immunoglobulin single variable domains (ISV), wherein one of the at least two ISVs is at the C-terminal end of the fusion protein, wherein the ISV at the C-terminal end of the fusion protein is a VHH, a humanized VHH, a VH, or a camelized VH that: does not bind to serum albumin; and has a C-terminal end of the sequence $VTVSS(X)_n$ (SEQ ID NO: 34), in which n is 1, 2, 3, 4, or 5, and in which each X is chosen from the group consisting of alanine (A), glycine (G), valine (V), leucine (L), and isoleucine (I), except with the proviso that when n is 3, each X is chosen from the group consisting of glycine (G), valine (V), leucine (L), and isoleucine (I), wherein the fusion protein does not comprise an ISV that binds IL-23 or other interleukins.

52.-53. (Canceled)

54. (Previously presented) The fusion protein according to claim 51, in which:
n = 1 or 2 in which each X = Ala or Gly; or
n = 1 or 2 in which each X = Ala; or
n = 1, 2 or 3 in which each X = Gly; or
n = 2 in which at least one X = Ala or Gly.

55. (Previously presented) The fusion protein according to claim 51, in which n = 1 or n = 2.

56. (Previously presented) A pharmaceutical composition comprising a fusion protein according to claim 51 and at least one suitable carrier, diluent or excipient.

CERTIFICATE OF COMPLIANCE

The foregoing filing complies with the relevant type-volume limitations and typeface and type style requirements of the Federal Rules of Appellate Procedure and Federal Circuit Rules because the filing has been prepared using a proportionally spaced typeface in 14-point Times New Roman font and includes 9,869 words, excluding the parts of the brief exempted by the Rules.

Dated: April 24, 2026

/s/ Daniel J. Minion
Daniel J. Minion