Anti-PCSK9~GLP-1 Fusions and Methods for Use

Field

[001] Anti-PCSK9~GLP-1 Fusions and Methods for Use

Background

[002] Diabetes is associated with higher cardiovascular morbidity and mortality. Hypertension, hyperlipidemia, and diabetes are independently associated with increased risk of cardiovascular disease. Subjects with Type 2 diabetes are at two- to four-fold increased risk of cardiovascular disease compared to those without diabetes.

[003] Glucagon-like peptide-1 (GLP-1) is known as a pleiotropic peptide with metabolic and cardiovascular benefits. It is derived from pre-proglucagon, a 158 amino acid precursor polypeptide that is processed in different tissues to form a number of different proglucagon-derived peptides, including glucagon, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2) and oxyntomodulin (OXM), that are involved in a wide variety of physiological functions, including glucose homeostasis, insulin secretion, gastric emptying, and intestinal growth, as well as the regulation of food intake. GLP-1 is produced as a 37-amino acid peptide that corresponds to amino acids 72 through 108 of proglucagon (92 to 128 of preproglucagon). The predominant biologically active form is a 30-amino acid peptide hormone (GLP-1(7-37) acid) that is produced in the gut following a meal and rapidly degraded by an abundant endogenous protease-DPP4. Baggio, L. and Drucker, D., Gasteroenterology, 132:2131-2157 (2007).

[004] GLP-1 and GLP-1 analogs, acting as agonists at the GLP-1 receptor, have been shown to be effective hypoglycemic control, e.g., type-2 diabetes. Certain GLP-1 analogs are being sold or are in development for treatment of type-2 diabetes including, *e.g.*, liraglutide (Victoza® from Novo Nordisk), dulaglutide (Eli Lilly), Bydureon (AZ/BMS), Aliblutide (GSK) and Exenatide (Byetta® from Eli Lilly/Amylin).

[005] One of the primary side effects following the initiation of GLP-1 therapy is gastrointestinal side effects, particularly nausea. This side effect is transient, resolves over time and can be mitigated by dose escalation. However, therapy is limited to patients that can tolerate the gastrointestinal side effects.

[006] PCSK9 is a nonenzymatic target for LDL cholesterol reduction and PCSK9 mutations correlate with reductions in LDL cholesterol and coronary heart disease. Cohen JC, *N Engl J Med*, 354:1264 (2006). PCSK9 antibodies have been shown to reduce LDL cholesterol in statin-treated patients and multiple candidates are undergoing clinical review.

[007] While there are a plurality of individual treatments for diabetes and cardiovascular diseases, there is a need for a single pharmaceutical composition to address both disease states (and the relationship between diabetes and cardiovascular disease). Providing a single pharmaceutical compound that has dual activities will reduce side effects, difficulties with patient compliance, and will increase beneficial outcomes to individual patients and will decrease costs incurred by the health care system.

SUMMARY

[008] In accordance with the description, disclosed is a dual active fusion molecule for the treatment of diabetes comprising an anti-PCSK9 antibody stably fused to a GLP-1 peptide, wherein the anti-PCSK9 antibody binds a PCSK9 polypeptide and the GLP-1 peptide binds a GLP-1 receptor.

[009] In one aspect, wherein the GLP-1 peptide is fused to the PCSK9 antibody via a linker peptide.

[010] In a further mode, the linker peptide is fused to the C-terminus of the GLP-1 peptide.

[011] In one embodiment, the GLP-1 peptide comprises the amino acid sequence of SEQ ID NO: 36.

[012] In one embodiment, the GLP-1 peptide comprises the amino acid sequence of SEQ ID NO: 3.

[013] In one mode, wherein the Cys18 of the GLP-1 molecule forms a disulfide bridge with the linker peptide or with the GLP-1 peptide itself.

[014] In one aspect, the fusion molecule controls glucose and/or reduces LDL in an animal. The animal may be human.

[015] Disclosed also is a dual active fusion molecule for the treatment of diabetes comprising an anti-PCSK9 antibody stably fused to a GLP-1 peptide comprising the amino acid sequence of SEQ ID NO: 3, wherein C-terminus of the GLP-1 peptide is fused via a peptide linker to the light chain of the anti-PCSK9 antibody, and wherein the anti-PCSK9 antibody binds a PCSK9 polypeptide and the GLP-1 peptide binds a GLP-1 receptor.

[016] Another embodiment disclosed is a method of treating Type 2 Diabetes comprising administering to a subject in need thereof, a fusion molecule described herein.

[017] A further aspect comprises administering to a subject in need thereof, a fusion molecule described herein.

[018] In one mode, a method of reducing low density lipoprotein (LDL) in a subject comprises administering to a subject in need thereof, a fusion molecule described herein.

[019] Another aspect encompasses a method of controlling glucose and reducing LDL in a subject comprising administering to a subject in need thereof, a fusion molecule described herein.

[020] Another aspect encompasses a method of promoting weight loss and reducing LDL in a subject comprising administering to a subject in need thereof, a fusion molecule described herein.

[021] In one embodiment, the subject has Type 2 diabetes.

[022] In another embodiment, the subject has metabolic syndrome.

[023] An additional aspect is a dual active fusion molecule comprising an antibody stably fused to a GLP-1 peptide comprising the amino acid sequence of SEQ ID NO: 3, wherein C-terminus of the GLP-1 peptide is fused via a peptide linker to the light chain of the antibody, and wherein the antibody binds a target polypeptide and the GLP-1 peptide binds a GLP-1 receptor.

[024] A further aspect is a dual active fusion molecule comprising an antibody stably fused to a GLP-1 peptide comprising the amino acid sequence of SEQ ID NO: 36, wherein C-terminus of the GLP-1 peptide is fused via a peptide linker to the light chain of the antibody, and wherein the antibody binds a target polypeptide and the GLP-1 peptide binds a GLP-1 receptor.

[025] In some aspects, the GLP-1 molecule comprises the amino acid sequence of SEQ ID NO: 36. In some aspects, the antibody is an anti-PCSK9 antibody.

[026] In certain modes, the light chain of the anti-PCSK9 antibody is at least 90% identical to the amino acid sequence of SEQ ID NO: 2. In other modes, the light chain of the anti-PCSK9 antibody comprises the amino acid sequence of SEQ ID NO: 2. In yet further modes, the heavy chain of the anti-PCSK9 antibody is at least 90% identical to the amino acid sequence of SEQ ID NO: 1. In some aspects, the heavy chain of the anti-PCSK9 antibody comprises the amino acid sequence of SEQ ID NO: 1.

[027] In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments of the dual active fusion molecule, the Cys18 of the GLP-1 molecule forms a disulfide bridge with the C terminus of the GLP-1 peptide.

[028] In some embodiments, the dual active fusion molecule is for the treatment of diabetes. In some embodiments, the dual active fusion molecule controls glucose and/or reduces LDL in an animal.

[029] In some modes, the animal is a human.

[030] Some embodiments include a dual active fusion molecule for the treatment of diabetes comprising an anti-PCSK9 antibody stably fused to a GLP-1 peptide that has reduced potency at the human GLP-1 receptor compared to a GLP-1 peptide comprising the amino acid sequence of SEQ ID NO: 29, wherein the C-terminus of the GLP-1 peptide is fused via a peptide linker to the anti-PCSK9 antibody, and wherein the anti-PCSK9 antibody binds a PCSK9 polypeptide and the GLP-1 peptide binds a GLP-1 receptor. In some embodiments, the GLP-1 peptide comprising the amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 29 is fused using a linker comprising SEQ ID NO:4 to the amino acid sequence comprising SEQ ID NO: 416. In further embodiments, the dual active fusion molecule of claims 25 or 26, wherein the potency at the human GLP- 1 receptor is reduced by 30 to 60 fold compared to a GLP-1 peptide comprising the amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 29.

[031] Some embodiments include an isolated polynucleotide encoding the fusion molecule described herein.

[032] In certain modes, encompassed is a vector comprising the polynucleotide described herein. In other modes, a host cell comprises the polynucleotide or vector described herein.

[033] In some embodiments, a method of making the fusion molecule comprises culturing the host cell under conditions allowing expression of the fusion molecule, and recovering the fusion molecule.

[034] In some modes, a pharmaceutical composition comprises the fusion molecule described herein and a carrier. In some modes, a kit comprises the composition described herein.

[035] Additional objects and advantages will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice. The objects

and advantages will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[036] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the claims.

[037] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate one (several) embodiment(s) and together with the description, serve to explain the principles described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[038] Figure 1A is a schematic for a dual action fusion molecule as described herein. Figures 1B-D show various schematics of certain embodiments of antibody-peptide fusion molecules.

[039] Figure 1E shows alignment using numerical numbering of PC9#2 variable heavy chain with germline sequence 1-46 (DP-7). Discrepancies are shown in white with a black background.

[040] Figure 1F shows alignment using numerical numbering of PC9#2 variable light chain with germline sequence VK1 O18 O8 (DPK1). Discrepancies are shown in white with a black background.

[041] Figures 2A-G show inhibition of human PCSK9 binding to anti-PCSK9 mAbs by anti-PCSK9 antibody/GLP-1 peptide fusions at the heavy chain N-terminus.

[042] Figures 3A-G show inhibition of human PCSK9 binding to anti-PCSK9 mAbs by anti-PCSK9 antibody/GLP-1 peptide fusions at the light chain N-terminus.

[043] Figures 4A-B show activation of human GLP1-Receptor by anti-PCSK9 antibody/GLP-1 peptide N-terminus fusions at the antibody heavy chain (A) and light chain (B).

[044] Figure 5A illustrates stability in rat for a GLP-1 analogue in heavy chain fusion with the control antibody NIP228.

[045] Figure 5B demonstrates stability in rat for an Exendin-4 GLP-1 analogue in light chain fusion with the anti-PCSK9 antibody PC9#2.

[046] Figure 5C demonstrates stability in mice for a GLP-1 analogue in fusion with human IgG4 Fc fragment.

[047] Figure 5D demonstrates stability in mice for a GLP-1 analogue in light chain fusion with the anti-PCSK9 antibody PC9#2.

[048] Figure 6 demonstrates a potential target profile to guide PCSK9 affinity and GLP-1 potency.

[049] Figure 7A provides peptide and linker amino acid sequences for eight compounds with an incorporated N-glycosylation consensus motif.

[050] Figure 7B provides peptide amino acid sequence for three compounds incorporating a disulphide bridge.

[051] Figure 8 shows a visual representation of the PC9#2_GLP1 molecule and the anti-PC9#2 antibody and GLP-1Fc(G4) used as a benchmark control.

[052] Figure 9 shows stability in rat of NIP228_GLP1_VH, a GLP-1 analogue in heavy chain fusion with the control antibody NIP228).

[053] Figure 10 shows stability in mice of PC9#2_GLP-1_VL, a GLP-1 analogue in light chain fusion with the anti-PCSK9 antibody PC9#2.

[054] Figure 11 shows stability in mice of PC9#2_DSB#3, Exendin-4 analogue DSB#3 in light chain fusion with the anti-PCSK9 antibody PC9#2.

[055] Figure 12 shows stability in mice of PC9#2_NGS#7, GLP-1 analogue NGS#7 in light chain fusion with the anti-PCSK9 antibody PC9#2.

[056] Figure 13A illustrates stability in mice for GLP-1 analogue NGS#7 in light chain fusion with the anti-PCSK9 antibody PC9#2.

[057] Figure 13B shows stability in mice for Exendin-4 analogue DSB#1 in light chain fusion with the anti-PCSK9 antibody PC9#2.

[058] Figure 13C illustrates stability in mice for Exendin-4 analogue DSB#3 in light chain fusion with the anti-PCSK9 antibody PC9#2.

[059] Figures 14A shows stability in mice for benchmark compound GLP-1-Fc fusion Open squares: concentration of test molecule in serum over time; open diamonds concentration of "active" test molecule (as measured by GLP-1 activity) over time for the same samples.

[060] Figures 14B shows stability in mice for Exendin-4 analogue DSB#1 in light chain fusion with the anti-PCSK9 antibody PC9#2. Open squares: concentration of test molecule in serum over time; open diamonds concentration of "active" test molecule (as measured by GLP-1 activity) over time for the same samples.

[061] Figure 15A provides a preparative SEC chromatogram for Exendin-4 analogue DSB#1 in light chain fusion with the anti-PCSK9 antibody PC9#2 after initial protein A purification.

[062] Figure 15B provides a preparative SEC chromatogram for Exendin-4 analogue DSB#3 in light chain fusion with the anti-PCSK9 antibody PC9#2 after initial protein A purification.

[063] Figure 16 shows an analytical SEC-HPLC profile of Exendin-4 analogue DSB#1 in light chain fusion with the anti-PCSK9 antibody PC9#2 after initial protein A purification.

[064] Figure 17 shows the amino acid sequence of additional Exendin-4 variant peptides incorporating a cysteine bridge. Cysteine residues are shown in black, other mutated residues are shown as underline and additional glycine residues at the C-terminus cap are shown in grey.

[065] Figure 18 provides a preparative SEC chromatogram for Exendin-4 analogue DSB#7 in light chain fusion with the anti-PCSK9 antibody PC9#2 after initial protein A purification.

[066] Figure 19 provides a preparative SEC chromatogram for Exendin-4 analogue DSB#9 in light chain fusion with the anti-PCSK9 antibody PC9#2 after initial protein A purification.

[067] Figure 20 shows stability in rat for Exendin-4 analogue DSB#7 in light chain fusion with the anti-PCSK9 antibody PC9#2.

[068] Figure 21 illustrates stability in rat for Exendin-4 analogue DSB#9 in light chain fusion with the anti-PCSK9 antibody PC9#2.

[069] Figure 22A shows simulation in human of the impact PCSK9/GLP-1 fusion for PCSK9 suppression.

[070] Figure 22B illustrates simulation in human of PCSK9/GLP-1 fusion molecule for GLP-1 agonism activity compared to Dulaglutide.

[071] Figure 23 shows the improved monomeric profile for PC9_2_DSB#7.

[072] Figure 24 illustrates the pharmacokinetic profile of peptide/antibody molecules in rats after a single i.v. injection.

[073] Figure 25 demonstrates inhibition of human PCSK9 binding to LDL receptor using competition ELISA.

[074] Figure 26 shows inhibition of human PCSK9-dependent loss of LDL uptake in HepG2 cells.

[075] Figure 27 shows the engineered reduction of potency at the human GLP-1 receptor.

[076] Figures 28A show a human GLP1r cAMP assay for Exendin-4 GLP-1 analogue DSB7_V19A in light chain fusion with the anti-PCSK9 antibody HS9.

[077] Figure 28B illustrates the stability in rat for Exendin-4 GLP-1 analogue DSB7_V19A in light chain fusion with the anti-PCSK9 antibody HS9.

[078] Figure 29A-D show specificity of the fusion molecule HS9_DSB7_V19 for GLP-1 receptor determined by cAMP assay.

[079] Figures 30A-B show superior glucose control (A) and weight loss (B) over time, including at day 7 post dose.

[080] Figure 31 shows binding to human PCSK9 of HS9 anti-PCSK9 antibody in fusion with GLP-1 analogue peptide DSB7_V19A using different linkers.

[081] Figure 32 shows human GLP-1 receptor activation using GLP-1 analogue peptide DSB7_V19A in fusion with anti-PCSK9 antibody HS9 using different linkers.

[082] Figure 33 illustrates binding to human PCSK9 of anti-PCSK9 antibodies in fusion with GLP-1 analogue peptide DSB7_V19A

[083] Figure 34 illustrates human GLP-1 receptor activation using GLP-1 analogue peptide DSB7_V19A in fusion with anti-PCSK9 antibodies

[084] Figure 35 demonstrates binding to human B7-H1 of anti-B7-H1 antibody 2.7A4 in fusion with GLP-1 analogue peptide DSB7_V19A

[085] Figure 36 shows human GLP-1 receptor activation using GLP-1 analogue peptide DSB7_V19A in fusion with anti-B7-H1 antibody 2.7A4.

[086] Figure 37 describes stability in rat for the fusion molecule HS9_DSB7_V19A following a single i.v. injection at 60, 30 or 10 mg/kg.

[087] Figure 38 shows free PCSK9 concentration in rat following a single i.v. injection at 60, 30, or 10 mg/kg of HS9_DSB7_V19A.

[088] Figure 39 shows total compound, active compound at GLP-1 receptor and free PCSK9 concentrations in rat following a single subcutaneous injection at 60 mg/kg of HS9_DSB7_V19A.

[089] Figures 40A-C provides the results from oral glucose tolerance tests (day 0 (A), day 2 (B), day 7 (C), confirming the ability of a single, subcutaneous administration of HS9_DSB7_V19A.

[090] Figure 41 shows body-weight change over time in the oral glucose tolerance test of Figures 40A-C.

[091] Figures 42A-C illustrates the results from oral glucose tolerance tests (day 0 (A), day 2 (B), day 7 (C).

[092] Figure 43 provides body-weight change over time in the oral glucose tolerance test of Figures 42A-C.

[093] Figure 44 shows body weight change in an intraperitoneal glucose tolerance test.

[094] Figure 45 demonstrates that weekly HS9_DSB7_V19A exhibited a dose dependent reduction in 4 hour fasting blood glucose compared to vehicle control in an intraperitoneal glucose tolerance test.

[095] Figure 46 shows that weekly dosed HS9_DSB7_V19A exhibited a dose dependent improvement in glucose tolerance as assessed by IPGTT at study day 22.

[096] Figures 47A-B shows blood glucose levels in a diet-induced obesity mouse model.

[097] Figures 48A-B shows body weight (grams) (A) and % change in body weight (B) in a diet-induced obesity mouse model.

[098] Figure 49 shows the change in body weight over time in a multiple dose study with HS9_DSB7_V19A.

[099] Figures 50A-B show the effect of the GLP-1 component of HS9_DSB7_V19A on glycemic control in a weekly dosing setting, measuring fed glucose (A) and terminal fasting glucose (B).

DESCRIPTION OF THE SEQUENCES

[0100] Table 1 provides a listing of certain sequences referenced in present embodiments.

Table 1

Description	Sequence	SEQ ID NO
	GROUP A	
anti-PCSK9 antibody heavy chain (HS9_VH)	QVQLVQSGAEVKKPGASVKVSCKASGYTFT**SYYMH**WVRQAPGQGLEWMG**EISPSGGSTSYNQKFQG**RVTMTRDT STSTVYMELSSLRSEDTAVYYCAR**ERPLYASDL**WGQGTTVTVSS	1.
anti-PCSK9 antibody light chain (HS9_Vk)	DIQMTQSPSSLSASVGDRVTITC**QASQDVKTAVA**WYQQKPGKAPKLLIY**SASYRYT**GVPSRFSGSGSGTDFTFT ISSLQPEDIATYYC**QQRYSLWRT**FGQGTKLEIK	2.
GLP-1 moiety with cysteine bridge and double mutation	*^ # HGEGTFTSDLSKQMEEE**CA**RLFIEWLKNGGPSSGAPPP**G**CG With the * designating a cysteine bridge to the linker, the ^ designating a point mutation to reduce potency and match CDTP (maximum efficacy without nausea side effect), and the # designating a point mutation to optimize disulfide bonding and reduce aggregation	3.
Linker	GGGGSGGGGSGGGGSA	4.

pH dependent version of anti-PCSK9 antibody heavy chain (PC9#2_FG_VH)	QVQLVQSGAEVKKPGASVKVSCKASGYTFT**SYYMH**WVRQAPGQGLEWMG**EIHPSGGSTSYNQKFQG**RVTMTRDT STSTVYMELSSLRSEDTAVYYCAR**ERPLYASDL**WGQGTTVTVSS	5.
pH dependent version of anti-PCSK9 antibody light chain (PC9#2_FG_Vk)	DIQMTQSPSSLSASVGDRVTITC**QASQDVHTAVA**WYQQKPGKAPKLLIY**HASYRYT**GVPSRFSGSGSGTDFTFT ISSLQPEDIATYYC**QQRYSLWRT**FGQGTKLEIK	6.
GLP-1 moiety with cysteine mutation to create disulfide bridge to linker	* HGEGTFTSDLSKQMEEE**C**VRLFIEWLKNGGPSSGAPPPSCG	7.
Antibody A pH dependent heavy chain (PC9_2_VH)	QVQLVQSGAEVKKPGASVKVSCKASGYTFT**SYYMH**WVRQAPGQGLEWMG**EIHPSGGRTNYNEKFKS**RVTMTRDT STSTVYMELSSLRSEDTAVYYCAR**ERPLYASDL**WGQGTTVTVSS	8.

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Antibody A pH dependent light chain (PC9_2_Vk)	DIQMTQSPSSLSASVGDRVTITC**KASQDVHTAVA**WYQQKPGKAPKLLIY**HASYRYT**GVPSRFSGSGSGTDFTFT ISSLQPEDIATYYC**QQRYSLWRT**FGQGTKLEIK	9.
Antibody B non pH dependent heavy chain (PC9_1_VH)	QVQLVQSGAEVKKPGASVKVSCKASGYTFT**SYYMH**WVRQAPGQGLEWMG**EISPFGGRTNYNEKFKS**RVTMTRDT STSTVYMELSSLRSEDTAVYYCAR**ERPLYASDL**WGQGTTVTVSS	10.
Antibody B non pH dependent light chain (PC9_1_Vk)	DIQMTQSPSSLSASVGDRVTITC**RASQGISSALA**WYQQKPGKAPKLLIY**SASYRYT**GVPSRFSGSGSGTDFTFT ISSLQPEDIATYYC**QQRYSLWRT**FGQGTKLEIK	11.
GLP-1 moiety (exenatide (Exe4))	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS	12.
GLP-1 moiety DSB#7 with linker (PC9_HS9_ DSB#7)	HGEGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSA	13.
anti-PCSK9 antibody heavy chain CDR1 (PC9_2_HS9)	**SYYMH**	14.

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anti-PCSK9 antibody heavy chain CDR2 (PC9_2_HS9_VH)	**EISPSGGSTSYNQKFQG**	15.
anti-PCSK9 antibody heavy chain CDR2 (PC9#2_FG_VH)	**EIHPSGGSTSYNQKFQG**	16.
anti-PCSK9 antibody heavy chain CDR2 (PC9_2_VH)	**EIHPSGGRTNYNEKFKS**	17.
anti-PCSK9 antibody heavy chain CDR2 (PC9_1_VH)	**EISPFGGRTNYNEKFKS**	18.
anti-PCSK9 antibody heavy chain CDR3	**ERPLYASDL**	19.
anti-PCSK9 antibody light chain CDR1 (PC9_2_HS9_Vk)	**QASQDVKTAVA**	20.

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anti-PCSK9 antibody light chain CDR1 (PC9#2_FG_Vk)	**QASQDVHTAVA**	21.
anti-PCSK9 antibody light chain CDR1 (PC9_2_Vk)	**KASQDVHTAVA**	22.
anti-PCSK9 antibody light chain CDR1 (PC9_1_Vk)	**RASQGISSALA**	23.
anti-PCSK9 antibody light chain CDR2 (PC9_2_HS9_Vk) (PC9_1_Vk)	**SASYRYT**	24.
anti-PCSK9 antibody light chain CDR2 (PC9#2_FG_Vk) (PC9_2_Vk)	**HASYRYT**	25.
anti-PCSK9 antibody light chain CDR3	**QQRYSLWRT**	26.

linker repeat	GGGGS	27.
GLP-1 moiety from dulaglutide (GLP-1 L)	HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG	28.
Human GLP-1 (7-37)	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG	29.
GLP-1 with disulfide Bridge (DSB) #1 DSB#1	HGEGTFTS**C**LSKQMEEEAVRLFIEWLKNGGPSSGAPPPSGGGGGGGGGGG**C**GG	30.
GLP-1 with DSB#2	HGE**C**TFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPSGGGGGGGGGGG**C**G	31.
GLP-1 with DSB#3	HGEGTFTSDLSKQMEEE**C**VRLFIEWLKNGGPSSGAPPG**C**	32.
GLP-1 with DSB#4	HGEGTFTSDLSKQMEEEAVR**C**FIEWLKNGGPSSGAGG**C**S	33.
GLP-1 with DSB#5	HGEGTFTS**C**LSKQMEEEAVRLFIEWLKNGGPSSGAPPPSGGGGGGGGG**C**GG	34.
GLP-1 with DSB#6	HGEGTFTS**C**LSKQMEEEAVRLFIEWLKNGGPSSGAPPPSGGGGGGGGGG**C**GGG	35.
GLP-1 with DSB#7	HGEGTFTSDLSKQMEEE**C**VRLFIEWLKNGGPSSGAPPPG**C**G	36.
GLP-1 with DSB#8	HGEGTFTSDLSKQMEEE**C**VRLFIEWLKNGGPSSGAPPGG**C**G	37.
GLP-1 with DSB#9	HGEGTFTSDLSKQMEEEAVR**C**FIEWLKNGGPSSGAPP**C**GG	38.
GLP-1 with DSB#10	HGEGTFTSDLSKQMEEEAVR**C**FIEWLKNGGPSSGAPPG**C**G	39.
GLP-1 with DSB#11	HGEGTFTSDLSKQMEEEAVRLFIE**C**LKNGGPSSGA**C**GGS	40.

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GLP-1 with DSB#12	HGEGTFTSDLSKQMEEEAVRLFIE**C**LKNGGPSSGAP**C**PS	41.
GLP-1 with DSB#13	HGEGTFTSDLSKQMEEEAVRLFIE**C**LKNGGPSSGAPP**C**S	42.
PC9#2_GLP1 VL with the linker of SEQ ID NO: 4 underlined (also referenced as PC9_2_GLP1)	HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVTIT**C**KASQ DVHTAVAWYQQKPGKAPKLLIYHASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYY**C**QQRYSLWRTFGQG TKLEIKR	43.
DSB#7 Variant G2V	H**V**EGTFTSDLSKQMEEE**C**VRLFIEWLKNGGPSSGAPPP**GCG**	44.
DSB#7 Variant E15A	HGEGTFTSDLSKQM**A**EE**C**VRLFIEWLKNGGPSSGAPPP**GCG**	45.
DSB#7 Variant L26I	HGEGTFTSDLSKQMEEE**C**VRLFIEW**I**KNGGPSSGAPPP**GCG**	46.
HS9_DSB7_V19A VL	***HGEGTFTSDLSKQMEEECARLFIEWLKNGGPSSGAPPPGCG***GGGGSGGGGSGGGGSADIQMTQSPSSLSASVGD RVTITC**QASQDVKTAVA**WYQQKPGKAPKLLIY**SASYRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQR YSLWRT**FGQGTKLEIK	47.
PC9_2_DSB#1 VL	***HGEGTFTSCLSKQMEEEAVRLFIEWLKNGGPSSGAPPPSGGGGGGGGGGGGCGG***GGGGSGGGGSGGGGSADIQMT QSPSSLSASVGDRVTITC**KASQDVHTAVA**WYQQKPGKAPKLLIY**HASYRYT**GVPSRFSGSGSGTDFTFTISSLQ PEDIATYYC**QQRYSLWRT**FGQGTKLEIKR	48.
PC9_2_DSB#3 VL	***HGEGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPGC***GGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRV TITC**KASQDVHTAVA**WYQQKPGKAPKLLIY**HASYRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQRYS LWRT**FGQGTKLEIKR	49.
PC9_2_NGS#7 VL	***HGEGTFTSDVSSYLEEQAAKEFIANLSKGGG***GGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVTITC**KASQ DVHTAVA**WYQQKPGKAPKLLIY**HASYRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQRYSLWRT**FGQG TKLEIKR	50.

PC9_2_DSB #7 VL	***HGEGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCG***GGGGSGGGGSGGGGSADIQMTQSPSSLSASVGD RVTITC**KASQDVHTAVA**WYQQKPGKAPKLLIY**HASYRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQR YSLWRT**FGQGTKLEIKR	51.
HS9_DSB# 7 VL	***HGEGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCG***GGGGSGGGGSGGGGSADIQMTQSPSSLSASVGD RVTITC**QASQDVKTAVA**WYQQKPGKAPKLLIY**SASYRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQR YSLWRT**FGQGTKLEIK	52.

GROUP B		
VH AB1 (pH-Dep)	QVQLVQSGAEVKKPGASVKVSCKASGYTFT**SYYMH**WVRQAPGQGLEWMG**EIHPSGGRTNYNEKFKS**RVTMTRDT STSTVYMELSSLRSEDTAVYYCAR**ERPLYASDL**WGQGTTVTVSS	53.
VL AB1 (pH-Dep)	DIQMTQSPSSLSASVGDRVTITC**KASQDVHTAVA**WYQQKPGKAPKLLIY**HASYRYT**GVPSRFSGSGSGTDFTFT ISSLQPEDIATYYC**QQRYSLWRT**FGQGTKLEIK	54.
VH AB2 L1L3	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTSY**YMHWVRQAPGQGLEWMGEI**SPFGGR**TNYNEKFKSRVTMTRDT STSTVYMELSSLRSEDTAVYYCAR**ERPLYASDL**WGQGTTVTVSS	55.
VL AB2 L1L3	DIQMTQSPSSLSASVGDRVTITC**RASQGISSALA**WYQQKPGKAPKLLIY**SASYRYT**GVPSRFSGSGSGTDFTFT ISSLQPEDIATYYC**QQRYSLWRT**FGQGTKLEIK	56.
VH AB3 4A5	EVQLQQSGPELVKPGASVKISCKAS**GYTFTDYYMN**WVKQSHGKSLEWIGDI**NPNNGG**TTYNQKFKGKATLTVDK SYSTAYMELRSLTSEDSAVYYCAR**WLLFAY**WGQGTLVTVSA	57.
VL AB3 4A5	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA**WYQQKPGQSPKALIY**SASYRY**SGVPDRFTGSGSGTDFTLT ISNVLSEDLAEYFC**QQFYSYPYT**FGGGTKLEIKR	58.
VH AB4 5A10	QVQLQQPGAELVKPGASVKLSCKAS**GYTFTSY**WMHWVKQRPGQGLEWIGEI**NPSNGR**TNYNEKFKSKATLTVDK SSSTAYMQLSSLTSEDSAVYYCARER**PLYAMDY**WGQGTSVTVSS	59.
VL AB4	DIVMTQSHKFMSTSVGDRVSITC**KASQDVSTAVA**WYQQKPGQSPKLLIY**SASYRY**TGVPDRFTGSGSGTDFTFT ISSVQAEDLAVYYC**QQRYSTPRT**FGGGTKLEIKR	60.

5A10		
VH AB4 6F6	EVQLQQSGPELVKPGASVKISCKAS**GYTFTDY**YMNWVKQSHGKSLEWIGDI**NPNNGG**TSYNQKFKGKATLTVDK SSSTAYMELRSLTSEDSAVYYCAGG**GIYYRYDRNYFDY**WGQGTTLTVSS	61.
VL AB4 6F6	DIQMTQTTSSLSASLGDRVTISC**SASQGISNYLN**WYQQKPDGTVKLLIY**YTSSLHS**GVPSRFSGSGSGTDYSLT ISNLEPEDIATYYC**QQYSKLPFT**FGSGTKLEIK	62.
VH AB4 7D4	EVKLVESEGGLVQPGSSMKLSCTASGFTFS**DYYMA**WVRQVPEKGLEWVA**NINYDGSNT**SYLDSLKSRFIISRDN AKNILYLQMSSLKSEDTATYYCAREKFA**AMDY**WGQGTSVTVSS	63.
VL AB4 7D4	DIVMTQSHKFMSTSFGDRVSITC**KASQDVSNALA**WYQQKPGHSPKLLIF**SASYRYT**GVPDRFTGSGSGTDFTFT ISSVQAEDLAVYYC**QQHYSTPWT**FGGGTKLEIKR	64.

GROUP C		
VH A74 30A4	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSYGMH**WVRQAPGKGLEWVA**VIWYDGSDKYYADSVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR**ETGLPKLYYYGMDV**WGQGTTVTVSS	65.
VH A85 3C4	QVQLQESGPGLVKPSQTLSLTCTVS**GGSISSSDYYWS**WIRQHPGKGLEWIG**YIYYSGSTYYNPSLKS**RITISVD TSKNLFSLKLSSVTAADTAVYYCAR**GGVTTYYYAMDV**WGQGTTVTVSS	66.
VH A71 23B5	EVQLLESGGGLVQPGGSLRLSCAAS**GFTFSSYAMN**WVRQAPGKGLEWVS**TISGSGDNTYYADSVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAK**KFVLMVYAMLDY**WGQGTLVTVSS	67.
VH A72 25G4	EVQLLESGGGLVQPGGSLRLSCAAS**GFTFSSYAMN**WVRQAPGKGLEWVS**TISGSGGNTYYADSVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAK**KFVLMVYAMLDY**WGQGTLVTVSS	68.
VH A67 31H4	EVQLVESGGGLVKPGGSLRLSCAAS**GFTFSSYSMN**WVRQAPGKGLEWVS**SISSSSSYISYADSVKG**RFTISRD NAKNSLYLQMNSLRAEDTAVYFCAR**DYDFWSAYYDAFDV**WGQGTMVTVSS	69.
VH A87 27B2	QVQLQESGPGLVKPSQTLSLTCTVS**GGSISSGGYYWS**WIRQHPGKGLEWIG**YIYNSGSTYYNPSLKS**RVTISVD TSKNQFSLKLSSVTAADTAVYYCAR**EDTAMVPYFDY**WGQGTLVTVSS	70.
VH	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFPSYGIS**WVRQAPGQGLEWMG**WISAYNGNTNYAEKLQG**RVTMTTDT	71.

A58 25A7	STSTAYMEVRSLRSDDTAVFYCAR**GYVMDV**WGQGTTVTVSS	
VH A52 27H5	QVQLVQSGAEVKRPGASVKVSCKAS**GYTLTSYGIS**WVRQAPGQGLEWMG**WISVYNGNTNYAQKVQG**RVTMTTDT STSTVYMELRSLSSDDTAVYYCAR**GYGMDV**WGQGTTVTVSS	72.
VH A51 26H5	QVQLVQSGAEVKKPGASVKVSCKAS**GYTLTSYGIS**WVRQAPGQGLEWMG**WISFYNGNTNYAQKVQG**RVTMTTDT STSTVYMELRSLRSDDTAVYYCAR**GYGMDV**WGQGTTVTVSS	73.
VH A53 31D1	QIQLVQSGAEVKKPGASVKVSCKAS**GYTLTSYGIS**WVRQAPGQGLEWMG**WISFYNGNTNYAQKVQG**RVTMTTDT STSTVYMELRSLRSDDTAVYFCAR**GYGMDV**WGQGTTVTVSS	74.
VH A48 20D10	QIQLVQSGAEVKKPGASVKVSCKAS**GYPLTSYGIS**WVRQAPGQGLEWMG**WISAYNGNTNYAQKVQG**SVTMTTDT STSTVYMELRSLRSDDTAVYYCAR**GYGMDV**WGQGTTVTVSS	75.
VH A54 27E7	QVQLVQSGAEVKKPGASLKVSCKAS**GYSLTSYGIS**WVRQAPGQGLEWMG**WISAYNGNTNYAQKVQG**RVTMTTDT STSTVYMEVRSLRSDDTAVYYCAR**GYGMDV**WGQGTTVTVSS	76.
VH A55 30B9	QVQLVQSGAEVKKPGASVKVSCKAS**GYPLTSYGIS**WVRQAPGQGLEWMG**WISAYNGNTNYAQKVQG**RVTMTTDT STSTVYMELRSLRSDDTAVYYCAR**GYGMDV**WGQGTTVTVSS	77.
VH A56 19H9	QVQLVQSGAEVKKPGASVKVSCKAS**GYALTSYGIS**WVRQAPGQGLEWMG**WISAYNGNTNYAQKVQG**RVTMTTDT STSTVYMELRSLRSDDTAVYYCAR**GYGMDV**WGQGTTVTVSS	78.
VH A49 21B12	QVQLVQSGAEVKKPGASVKVSCKAS**GYTLTSYGIS**WVRQAPGQGLEWMG**WVSFYNGNTNYAQKLQG**RGTMTTDP STSTAYMELRSLRSDDTAVYYCAR**GYGMDV**WGQGTTVTVSS	79.
VH A57 17C2	QVQLVQSGAEVKKPGASVKVSCKAS**GYSFTSYGIS**WVRQAPGQGLEWMG**WVSAYNGNTNYAQKFQG**RVTMTTDT STSTAYMELRSLRSDDTAVYYCAR**GYVMDV**WGQGTTVTVSS	80.
VH A50 23G1	QVQLVQSGAEVKKPGASVKVSCKAS**GYTLTSYGIS**WVRQAPGQGLEWMG**WVSFYNGNTNYAQKLQG**RGTMTTDP STSTAYMELRSLRSDDTAVYYCAR**GYGMDV**WGQGTTVTVSS	81.
VH A91	QVQLQQSGPGLVKPSQTLSLTCAIS**GDSVSSNSAAWN**WIRQSPSRGLEWLG**RTYYRSKWYKNYSVSVKS**RITIN PDTSKNQFSLQLNSVTPGDTAVYYCAR**GGPTAAFDY**WGQGTLVTVSS	82.

13H1		
VH A64 9C9	EVQLVESGGGLVQPGGSLRLSCVVS**GFTFSSYWMS**WVRQAPGKGLEWVA**NIKQDGSEKYYVDSVKG**RFTISRDN AKNSLYLQMNSLRAEDTAVYYCAR**ESNWGFAFDI**WGQGTMVTVSS	83.
VH A62 9H6	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFSRYWMS**WVRQAPGKGLEWVA**NIKHDGSEKYYVDSVKG**RFTISRDN AKNSLYLQMNSLRAEDTAVYYCAR**ESNWGFAFDV**WGHGTMVTVSS	84.
VH A89 31A4	QVQLQQWGAGLLKPSETLSLTCAVY**GGSFSAYYWN**WIRQPPGKGLEWIG**EINHSGRTDYNPSLKS**RVTISVDTS KKQFSLKLNSVTAADTAVYYCAR**GQLVPFDY**WGQGTLVTVSS	85.
VH A65 1A12	EVQLVESGGGLVQPGGSLRLSCAAS**GLTFSNFWMS**WVRQAPGKGLEWVA**NIKQDGSEKYYVDSVKG**RFTISRDN AKNSLYLQMNSLRAEDTAVYSCTR**ESNWGFAFDI**WGQGTMVTVSS	86.
VH A79 16F12	QVHLVESGGGVVQPGRSLRLSCAAS**GFTFNSFGMH**WVRQAPGKGLEWVA**LIWSDGSDEYYADSVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR**AIAALYYYYGMDV**WGQGTTVTVSS	87.
VH A80 22E2	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSFGMH**WVRQAPGKGLEWVA**LIWNDGSNKYYADSVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR**AIAALYYYYGMDV**WGQGTTVTVSS	88.
VH A76 27A6	QVHLVESGGGVVQPGRSLRLSCAAS**GFTFNSFGMH**WVRQAPGKGLEWVA**LIWSDGSDKYYADSVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR**AIAALYYYYGMDV**WGQGTTVTVSS	89.
VH A77 28B12	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSFGMH**WVRQAPGKGLEWVA**LIWNDGSNKYYADSVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR**AIAALYYYYGMDV**WGHGTTVTVSS	90.
VH A78 28D6	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSFGMH**WVRQAPGKGLEWVA**LIWNDGSNKYYADSVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR**AIAALYYYYGMDV**WGQGTTVTVSS	91.
VH A83 31G11	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFRSYGMH**WVRQAPGKGLEWVA**LIWHDGSNTYYVDSVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR**GIAVAYYYYGMDV**WGQGTTVTVSS	92.
VH A69 13B5	EVQLLESGGGLVQPGGSLRLSCAAS**GFTFSSYAMS**WVRQAPGKGLEWVS**TISGSGGRTYYADSVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAK**EVGSPFDY**WGQGTLVTVSS	93.

VH A81 31B12	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSYGMH**WVRQAPGKGLEWVA**IIWYDGSNKYYADSVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR**RGGLAARPGGMDV**WGQGTTVTVSS	94.
VH A60 3B6	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTSYGIS**WVRQAPGQGLEWMG**WISTYNGNTNYAQKVQG**RVTMTTDT STSTAYMELRSLRSDDTAVYYCAR**GYTRDY**WGQGTLVTVSS	95.
VL 5 30A4	DIVMTQSPLSLSVTPGEPPSISC**RSSQSLLHSNGYNFLN**WYLQKPGQSPQLLIY**LGSHRAS**GVPDRFSGSGSGT DFTLEISRVEAEDVGVYYC**MQVLQTPFT**FGPGTKVDIK	96.
VL 7 3C4	DIQMTQSPSSLSASVGDRVTITC**RASQRISNYLS**WYLQKPGIAPKLLIY**AASSLQS**GVPSRFSGSGSGTDFTLT ISSLQSEDFATYYC**QQSYSTPLI**FGGGTKVEIK	97.
VL 9 23B5	DILMTQSPSSLSASVGDRVTITC**RASQSISSYLN**WYQQKPGKAPKVLIY**AASSLQS**GVPSRFSGSGSGTDFTLT INSLQPEDFATYYC**QQSYSSPIT**FGQGTRLEIK	98.
VL 10 25G4	DIQMTQSPSSLSASVGDRVTITC**RASQSISIYLN**WYQQKPGKAPYLLIY**AAASLQS**GVPSRFSGSGSGTDFTLT ISSLQPEDFATYYC**QQSYSAPIT**FGQGTRLEIK	99.
VL 12 31H4	QSVLTQPPSVSGAPGQRVTISC**TGSSSNIGAGYDVH**WYQQLPGTAPKLLIS**GNSNRPS**GVPDRFSGSKSGTSAS LAITGLQAEDEADYYC**QSYDSSLSGSV**FGGGTKLTVL	100.
VL 13 27B2	QSVLTQPPSVSGAPGQRVTISC**TGSSSNIGAHYDVH**WYQQVPGTAPKLLIY**GNTYRPS**GVPDRFSGSKSGTSAS LAITGLQAEDEADYYC**QSYDNSLSGVV**FGGGTKLTVL	101.
VL 15 25A7	QSALTQPASVSGSPGQSITISC**TGTSSDVGRYNSVS**WYQHHPGKAPKVMIY**EVSNRPS**GVSTRFSGSKSGNTAS LTISGLQAEDEADYYC**SSYTSSSVV**FGGGTKLTVL	102.
VL 16 27H5	QSALTQPASVSGSPGQSITISC**TGTSSDVGGYNSVS**WYQQHPGKPPKLMIY**EVSNRPS**GVSIRFSGSKSGNTAS LTISGLQAEDEADYFC**SSYTSTSMV**FGGGTKLTVL	103.
VL 17 26H5	QSALTQPASVSGSPGQSITISC**TGTSSDVGGYNSVS**WYQQHPGKPPKLMIY**EVSNRPS**GVSIRFSGSKSGNTAS LTISGLQAEDEADYFC**SSYTSTSMV**FGGGTKLTVL	104.
VL	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNSVSWYQQHPGKPPKLMIY**EVSNRPS**GVSNRFSGSKSGNTAS	105.

18 31D1	LTISGLQAEDEADYFC**SSYTSTSMV**FGGGTKLAVL	
VL 19 20D10	QSALTQPASVSGSPGQSITISC**TGTSSDVGGYNSVS**WYQQYPGKPPKLKIY**EVSNRPS**GVSNRFSGSKSGNTAS LTISGLQAEDEADYFC**SSYTSTSMV**FGGGTKLTVL	106.
VL 20 27E7	QSALTQPASVSGSPGQSITISC**TGTSSDVGGYNSVS**WYQQHPGKPPKLMIY**EVSNRPS**GVSNRFSGSKSGNTAS LTISGLQAEDEADYFC**SSYTSTSMV**FGGGTKLTVL	107.
VL 21 30B9	QSALTQPASVSGSPGQSITISC**TGTSSDVGGYNSVS**WYQQHPGKPPKLMIY**EVSNRPS**GVSNRFSGSKSGNTAS LTISGLQAEDEADYFC**SSYTSTSMV**FGGGTKLTVL	108.
VL 22 19H9	QSALTQPASVSGSPGQSITISC**TGTNSDVGGYNSVS**WYQQHPGKPPKLMIY**EVSNRPS**GISNRFSGSKSGNTAS LTISGLQAEDEADYFC**SSYTSTSMV**FGGGTKLTVL	109.
VL 23 21B12	QSALTQPASVSGSPGQSITISC**TGTSSDVGGYNSVS**WYQQHPGKAPKLMIY**EVSNRPS**GVSNRFSGSKSGNTAS LTISGLQAEDEADYYC**NSYTSTSMV**FGGGTKLTVL	110.
VL 24 17C2	QSALTQPASVSGSPGQSITISC**TGTSSDVGAYNSVS**WYQQHPGKAPKRMIY**EVSNRPS**GVSNRFSGSKSGNTAS LTISGLQAEDEADYYC**SSYTSTNMV**FGGGTKLTVL	111.
VL 26 23G1	QSALTQPASVSGSPGQSITISC**TGTSSDVGGYNSVS**WYQQHPGKAPKLMIY**EVTNRPS**GVSNRFSGSKSGNTAS LTISGLQAEDEADYYC**NSYTSTSMV**FGGGTKLTVL	112.
VL 28 13H1	LSALTQPASVSGSPGQSITISC**TGTSSDVGNYNLVS**WYQQYSGKAPKLMIY**EVSKRPS**GVSNRFSGSKSGNTAS LTISGLQAEDEADYYC**CSYAGSSTLV**FGGGTKLTVL	113.
VL 30 9C9	QSVLTQPPSASGTPGQRVTISC**SGSSSNIGSKTVN**WYQQVPGTAPKLLIY**RNNQRPL**GVPDRFSGSKSGTSASL AISGLQSEDEADYYC**AAWDDSLNWV**FGGGTKLTVL	114.
VL 31 9H6	QSVLTQPPSASGPPGQRVTISC**SGSSSNIGSNTVN**WYQQLPGTAPKLLIY**SNNRRPS**GVPDRFSGSKSGTSASL AISGLQSEDEADYYC**AAWDDSLNWV**FGGGTKLTVL	115.
VL 32	QSVLTQPPSASGTPGQRVTISC**SGSSSNIGSNTVN**WYQQLPGTAPKLLIY**SNNQRPS**GVPDRFSGSKSGTSASL AISGLQSEDEADYYC**AVWDDSLNGWV**FGGGTKLTVL	116.

31A4		
VL 33 1A12	QSVLTQPPSASGTPGQRVTISC**SGSSSNIGSKTVN**WYQQFPGTAPKLLIY**SNNRRPS**GVPDRFSGSKSGTSASL AISGLQSEDEADYYC**AAWDDSLNWV**FGAGTKLTVL	117.
VL 35 16F12	QSVLTQPPSVSAAPGQKVTISC**SGSSSNIGNNFVS**WYQQLPGTAPKLLIY**DYNKRPS**GIPDRFSGSKSGTSATL GITGLQTGDEADYYC**GTWDSSLSAYV**FGTGTRVTVL	118.
VL 36 22E2	QSVLTQPPSVSAAPGQKVTISC**SGSSSNIGNNFVS**WYQQLPGTAPKLLIY**DYNKRPS**GIPDRFSGSKSGTSATL GITGLQTGDEADYYC**GTWDSSLSGYV**FGTGTRVTVL	119.
VL 37 27A6	QSVLTQPPSVSAAPGQKVTISC**SGSSSNIGNNFVS**WYQQFPGTAPKLLIY**DYNKRPS**GIPDRFSGSKSGTSATL GITGLQTGDEADYYC**GTWDSSLSSYV**FGTGTRVTVL	120.
VL 38 28B12	QSVLTQPPSVSAAPGQKVTISC**SGSSSNIGNNFVS**WYQQLPGTAPKLLIY**DYNKRPS**GIPDRFSGSKSGTSATL GITGLQTGDEADYYC**GTWDSSLSGYV**FGTGTRVTVL	121.
VL 39 28D6	QSVLTQPPTVSAAPGQKVTISC**SGSSSNIGNNFVS**WYQQLPGTAPKLLIY**DYNKRPS**GIPDRFSGSKSGTSATL GITGLQTGDEADYYC**GTWDSSLSGYV**FGTGTRVTVL	122.
VL 40 31G11	QSVLTQPPSVSAAPGQKVTISC**SGSSSNIGNNFVS**WYQQLPGTAPKLLIY**DSNKRPS**GIPDRFSGSKSGTSATL DITGLQTGDEADYYC**GTWDSSLSAYV**FGTGTKVTVL	123.
VL 42 13B5	QSVLTQPPSVSAAPGQKVTISC**SGSNSNIGNNYVS**WYQQLPGTAPKLLIY**DNNKRPS**GIPDRFSGSNSGTSATL GITGLQTGDEADYYC**GTWDSSLSAVV**FGGGTKLTVL	124.
VL 44 31B12	SYELTQPPSVSVSPGQTARITC**SGDKLGDKYAC**WYQQKPGQSPVLVIY**QNTKWPL**GIPERFSGSKSGNTVTLTI SGTQAMDEADYYC**QAWDSSTVV**FGGGTKLTVL	125.
VL 46 3B6	QPVLTQPLFASASLGASVTLTC**TLSSGYSSYEVD**WYQQRPGKGPRFVMR**VDTGGIVGSKGE**GIPDRFSVLGSGL NRYLTIKNIQEEDESDYHC**GADHGSGTNFVVV**FGGGTKLTVL	126.

GROUP D		
VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSYG**MHWVRQAPGKGLEWVAF**IGFDGSNI**YYGDSVRGRIIISRDN	127.

66	SENTLYLEMNSLRAEDTAVYYC**AREKGLD**WGQGTLVTVSS	
VH 90 H1H316P	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFNNYA**MNWVRQAPGKGLDWVST**ISGSGGTT**NYADSVKGRFIISRDS SKHTLYLQMNSLRAEDTAVYYC**AKDSNWGNFDL**WGRGTLVTVSS	128.
VH 138	QVQLQESGPGLVKPSETLSLTCTVS**GDSINTYY**WSWFRQPPGKGLEWIGY**IYYSGTT**NYNPSLKSRVTISIDTP RNQFSLKLISVTAADTAVYYC**ARERITMIRGVTLYYYSYGMDV**WGQGTTVTVSS	129.
VH 218 H1M300N	EMQLVESGGGLVQPGGSLRLSCAAS**GFTFSSHW**MKWVRQAPGKGLEWVAN**INQDGSEK**YYVDSVKGRFTISRDN AKNSLFLQMNSLRAEDTAVYYC**ARDIVLMVYDMDYYYYGMDV**WGQGTTVTVSS	130.
VH 2	QVQLVQSGGGLVQPGGSLRLSCAAS**GFTLSSYD**MHWVRQSTGKGLEWVSA**IGSTGDT**YYPGSVKGRFTITREKA KNSVYLQMNSLRAGDTAVYYC**VREGWEVPFDY**WGQGTLVTVSS	131.
VH 18	EVQLVESGGGLVQPGGSLRLSCAAS**GFTLSSYD**MHWVRQSTGKGLEWVSA**IGSTGDT**YYPGSVKGRFTITREKA KNSVYLQMNSLRAGDTAVYYC**VREGWEVPFDY**WGQGTLVTVSS	132.
VH 22	EVQLVESGGGLVQPGGSLRLSCAAS**GFTLSSYD**MHWVRQATGKGLEWVSA**IGSTGDT**YYPGSVKGRFTISRENA KNSLYLQMNSLRAGDTAVYYC**VREGWEVPFDY**WGQGTLVTVSS	133.
VH 26	QVQLVQSGGGVVQPGRSLRLSCAAS**GFTFSSYG**MHWVRQAPGKGLEWVAF**IGFDGSNI**HYGDSVRGRIIISRDN SENTLYLEMNSLRAEDTAMYYC**AREKGLD**WGQGTTVTVSS	134.
VH 42	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSYG**MHWVRQAPGKGLEWVAF**IGFDGSNI**HYGDSVRGRIIISRDN SENTLYLEMNSLRAEDTAMYYC**AREKGLD**WGQGTLVTVSS	135.
VH 46	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSYG**MHWVRQAPGKGLEWVAV**IGFDGSNI**YYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC**AREKGLD**WGQGTLVTVSS	136.
VH 50	QVQLQESGGGVVQPGRSLRLSCAAS**GFTFSSYG**MHWVRQAPGKGLEWVAF**IGFDGSNI**YYGDSVRGRIIISRDN SENTLYLEMNSLRAEDTAVYYC**AREKGLD**WGQGTLVTVSS	137.
VH 70	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSYG**MHWVRQAPGKGLEWVAV**IGFDGSNI**YYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC**AREKGLD**WGQGTLVTVSS	138.
VH 94	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFNNYA**MSWVRQAPGKGLEWVSA**ISGSGGTT**YYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC**AKDSNWGNFDL**WGRGTLVTVSS	139.
VH 98	QVQLVQSGGGLVQPGGSLRLSCAVS**GFTLSSYD**MHWVRQPTGKGLEWVSA**IGSTGDT**YYPGSVKGRFTISRENA KNSLYLQMNSLRAGDTAVYYC**AREGWDVPFDF**WGQGTLVTVSS	140.
VH 114	EVQLVESGGGLVQPGGSLRLSCAVS**GFTLSSYD**MHWVRQPTGKGLEWVSA**IGSTGDT**YYPGSVKGRFTISRENA KNSLYLQMNSLRAGDTAVYYC**AREGWDVPFDF**WGQGTLVTVSS	141.
VH 118	EVQLVESGGGLVQPGGSLRLSCAAS**GFTLSSYD**MHWVRQATGKGLEWVSA**IGSTGDT**YYPGSVKGRFTISRENA KNSLYLQMNSLRAGDTAVYYC**AREGWDVPFDF**WGQGTLVTVSS	142.

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VH 122	QVQLQESGPGLVKPSETLSLTCTVS**GDSINTYY**WSWFRQPPGKGLEWIGY**IYYSGTT**NYNPSLKSRVTISIDTP RNQFSLKLISVTAADTAVYYC**ARERITMIRGVTLYYYSYGMDV**WGQGTTVTVSS	143.
VH 142	QVQLQESGPGLVKPSETLSLTCTVS**GDSINTYY**WSWIRQPPGKGLEWIGY**IYYSGTT**NYNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYC**ARERITMIRGVTLYYYSYGMDV**WGQGTTVTVSS	144.
VH 146	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTNYG**ISWVRQAPGQGLELMGW**ISGYNGNT**NYAQELQARVTMTTDT STSTAYMELRNLRSDDTAVYYC**ARDRVVVAAANYYFYSMDV**WGQGTTVTVSS	145.
VH 162	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTNYG**ISWVRQAPGQGLELMGW**ISGYNGNT**NYAQELQARVTMTTDT STSTAYMELRNLRSDDTAVYYC**ARDRVVVAAANYYFYSMDV**WGQGTTVTVSS	146.
VH 166	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTNYG**ISWVRQAPGQGLEWMGW**ISGYNGNT**NYAQKLQGRVTMTTDT STSTAYMELRSLRSDDTAVYYC**ARDRVVVAAANYYFYSMDV**WGQGTTVTVSS	147.
VH 170	QVHLKESGPTLVKPTQTLTLTCTFS**GFSLITSGVG**VGWIRQPPGKALEWLAL**IYWNGDK**RYSPSLKSRLTITKD TSKNQVVLTMTNMDPVDTATYYC**AHRITETSYYFYYGMDV**WGQGTTVTVSS	148.
VH 186	QITLKESGPTLVKPTQTLTLTCTFS**GFSLITSGVGVG**WIRQPPGKALEWLAL**IYWNGDK**RYSPSLKSRLTITKD TSKNQVVLTMTNMDPVDTATYYC**AHRITETSYYFYYGMDV**WGQGTTVTVSS	149.
VH 190	QITLKESGPTLVKPTQTLTLTCTFS**GFSLITSGVGVG**WIRQPPGKALEWLAL**IYWNGDK**RYSPSLKSRLTITKD TSKNQVVLTMTNMDPVDTATYYC**AHRITETSYYFYYGMDV**WGQGTTVTVSS	150.
VH 194	QITLKESGPTLVKPSQTLTLTCTFS**GFSLSTSGVG**VGWIRQPPGKALEWLAL**IYWNSDK**RYSPSLKSRLTITKD TSKNQVVLTMTNMDPVDTATYYC**AHRHDSSSYYFYYGMDV**WGQGITVTVSS	151.
VH 210	QITLKESGPTLVKPSQTLTLTCTFS**GFSLSTSGVG**VGWIRQPPGKALEWLAL**IYWNSDK**RYSPSLKSRLTITKD TSKNQVVLTMTNMDPVDTATYYC**AHRHDSSSYYFYYGMDV**WGQGTTVTVSS	152.
VH 214	QITLKESGPTLVKPTQTLTLTCTFS**GFSLSTSGVG**VGWIRQPPGKALEWLAL**IYWNSDK**RYSPSLKSRLTITKD TSKNQVVLTMTNMDPVDTATYYC**AHRHDSSSYYFYYGMDV**WGQGTTVTVSS	153.
VH 234	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFSSHW**MKWVRQAPGKGLEWVAN**INQDGSEK**YYVDSVKGRFTISRDN AKNSLFLQMNSLRAEDTAVYYC**ARDIVLMVYDMDYYYYGMDV**WGQGTTVTVSS	154.
VH 238	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFSSHW**MSWVRQAPGKGLEWVAN**INQDGSEK**YYVDSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYC**ARDIVLMVYDMDYYYYGMDV**WGQGTTVTVSS	155.
VH 242	QVQLVESGGGVVQPGRSLRLSCAVS**GFTFSSYG**MHWVRQAPGKGLEWVAA**ISYDGSNK**YYVDSVKGRFTISRDN SKKTLYLQMNSLRAEDTAVYNC**AKNIVLVMYDIDYHYYGMDV**WGQGTTVTVSS	156.
VH 258	QVQLVESGGGVVQPGRSLRLSCAVS**GFTFSSYG**MHWVRQAPGKGLEWVAA**ISYDGSNK**YYVDSVKGRFTISRDN SKKTLYLQMNSLRAEDTAVYNC**AKNIVLVMYDIDYHYYGMDV**WGQGTTVTVSS	157.
VH 262	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSYG**MHWVRQAPGKGLEWVAV**ISYDGSNK**YYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC**AKNIVLVMYDIDYHYYGMDV**WGQGTTVTVSS	158.
VH	QVQLVESGGGVVQPGRSLRLSCAVS**GFTFSSYG**MHWVRQAPGKGLEWVAA**ISYDGSNK**YYVDSVKGRFTISRDN	159.

266	SKKTLYLQMNSLRAEDTAVYNC**AKNIVLVMYDIDYHYYGMDV**WGQGTTVTVSS	
VH 282	QVQLVESGGGVVQPGRSLRLSCAVS**GFTFSSYG**MHWVRQAPGKGLEWVAA**ISYDGSNK**YYVDSVKGRFTISRDN SKKTLYLQMNSLRAEDTAVYNC**AKNIVLVMYDIDYHYYGMDV**WGQGTTVTVSS	160.
VH 286	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSYG**MHWVRQAPGKGLEWVAV**ISYDGSNK**YYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC**AKNIVLVMYDIDYHYYGMDV**WGQGTTVTVSS	161.
VH 290	QITLKESGPTLVKPTQTLTLTCTFS**GFSLSASGVG**VGWFRQPPGKALEWLAL**IYWNDDK**RYSPSLKNSLTITKD TSKNQVVLTMTNMDPVDTATYYC**AHRIHLWSYFYYGMDV**WGQGTTVTVSS	162.
VH 306	QITLKESGPTLVKPTQTLTLTCTFS**GFSLSASGVG**VGWFRQPPGKALEWLAL**IYWNDDK**RYSPSLKNSLTITKD TSKNQVVLTMTNMDPVDTATYYC**AHRIHLWSYFYYGMDV**WGQGTTVTVSS	163.
VH 310	QITLKESGPTLVKPTQTLTLTCTFS**GFSLSASGVG**VGWIRQPPGKALEWLAL**IYWNDDK**RYSPSLKSRLTITKD TSKNQVVLTMTNMDPVDTATYYC**AHRIHLWSYFYYGMDV**WGQGTTVTVSS	164.
VH 314	QVQLVQSGPEVKNPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NDAQKFQDRVAMTTDT STSTAYMELRSLRSDDTAIYYC**SRDRLVVPPALNYSYYVMDV**WGQGTTVTVSS	165.
VH 330	QVQLVQSGPEVKNPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NDAQKFQDRVAMTTDT STSTAYMELRSLRSDDTAIYYC**SRDRLVVPPALNYSYYVMDV**WGQGTTVTVSS	166.
VH 334	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NYAQKLQGRVTMTTDT STSTAYMELRSLRSDDTAVYYC**SRDRLVVPPALNYSYYVMDV**WGQGTTVTVSS	167.
VH 338	EVQLVESGGGLVKPGGSLRLSCAAS**GFTFSSYS**MDWVRQAPGKGLEWVSS**ISSSSSYI**YYADSVKGRFTISRDT AKNSLYLQMNSLRDEDTAVYYC**AREGSSRLFDY**WGQGTLVTVSS	168.
VH 354	EVQLVESGGGLVKPGGSLRLSCAAS**GFTFSSYS**MDWVRQAPGKGLEWVSS**ISSSSSYI**YYADSVKGRFTISRDT AKNSLYLQMNSLRDEDTAVYYC**AREGSSRLFDY**WGQGTLVTVSS	169.
VH 358	EVQLVESGGGLVKPGGSLRLSCAAS**GFTFSSYS**MNWVRQAPGKGLEWVSS**ISSSSSYI**YYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYC**AREGSSRLFDY**WGQGTLVTVSS	170.
VH 362	QVHLVESGGGLVKPGGSLRLSCAAS**GFTFSDHY**MSWIRQAPGKGLEWISY**ISNDGGTK**YYVDSVEGRFIISRDN AKNSLYLHMNSLRADDTAVYYC**ARDQGYIGYDSYYYYSYGMDV**WGQGTTVTVAS	171.
VH 378	QVQLVESGGGLVKPGGSLRLSCAAS**GFTFSDHY**MSWIRQAPGKGLEWISY**ISNDGGTK**YYVDSVEGRFIISRDN AKNSLYLHMNSLRADDTAVYYC**ARDQGYIGYDSYYYYSYGMDV**WGQGTTVTVSS	172.
VH 382	QVQLVESGGGLVKPGGSLRLSCAAS**GFTFSDHY**MSWIRQAPGKGLEWVSY**ISNDGGTK**YYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYC**ARDQGYIGYDSYYYYSYGMDV**WGQGTTVTVSS	173.
VH 386	EVQKVESGGGLVKPGGSLRLSCTAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKNSLYLQMNSLRADDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	174.
VH 402	EVQLVESGGGLVKPGGSLRLSCTAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKNSLYLQMNSLRADDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	175.

VH 406	EVQLVESGGGLVKPGGSLRLSCAAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	176.
VH 410	EVQLVESGGGLVKPGGSLRLSCTAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKNSLYLQMNSLRADDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	177.
VH 426	EVQLVESGGGLVKPGGSLRLSCTAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKNSLYLQMNSLRADDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	178.
VH 430	EVQLVESGGGLVKPGGSLRLSCAAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	179.
VH 434	EVQLVESGGGLVKPGGSLRLSCTAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKSSLYLQMNSLRAEDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	180.
VH 450	EVQLVESGGGLVKPGGSLRLSCTAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKSSLYLQMNSLRAEDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	181.
VH 454	EVQLVESGGGLVKPGGSLRLSCAAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	182.
VH 458	EVQLVESGGGLVKPGGSLRLSCTAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKNSLYLQMNSLRADDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	183.
VH 474	EVQLVESGGGLVKPGGSLRLSCTAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKNSLYLQMNSLRADDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	184.
VH 478	EVQLVESGGGLVKPGGSLRLSCAAS**GFTFSTYN**MNWVRQAPGKGLEWVSS**IRSSSNYI**YYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYC**ARDGSSWYDYSDY**WGQGTLVTVSS	185.
VH 482	EVQLVESGGGLVQPGGSLRLSCVVS**GFTFGDYD**MHWVRQATGRGLEWVSG**IAPAGDT**SYTGSVKGRFTISRENA KNSLHLQMNSLTTGDTAIYYC**AREDIAVPGFDY**WGQGTLVTVSS	186.
VH 498	EVQLVESGGGLVQPGGSLRLSCVVS**GFTFGDYD**MHWVRQATGRGLEWVSG**IAPAGDT**SYTGSVKGRFTISRENA KNSLHLQMNSLTTGDTAIYYC**AREDIAVPGFDY**WGQGTLVTVSS	187.
VH 502	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFGDYD**MHWVRQATGKGLEWVSA**IAPAGDT**YYPGSVKGRFTISRENA KNSLYLQMNSLRAGDTAVYYC**AREDIAVPGFDY**WGQGTLVTVSS	188.
VH 506	QILLVQSGPEVKEPGASVKVSCKAS**GYTFTNYA**ISWVRQVPGQGLEWMGW**VSAYNGHT**NYAHEVQGRVTMTTDT STTTAYMELRSLRSDDTAMYYC**ARGGVVVPVAPHFYNGMDV**WGQGTTVTVSS	189.
VH 522	QVQLVQSGPEVKEPGASVKVSCKAS**GYTFTNYA**ISWVRQVPGQGLEWMGW**VSAYNGHT**NYAHEVQGRVTMTTDT STTTAYMELRSLRSDDTAMYYC**ARGGVVVPVAPHFYNGMDV**WGQGTTVTVSS	190.
VH 526	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTNYA**ISWVRQAPGQGLEWMGW**VSAYNGHT**NYAQKLQGRVTMTTDT STSTAYMELRSLRSDDTAVYYC**ARGGVVVPVAPHFYNGMDV**WGQGTTVTVSS	191.
VH	EVQLVESGGGLVQPGGSLRLSCAAS**GFTLSSYD**MHWVRQATGKGLEWVSA**IGSTGDT**YYTGSVMGRFTISRDAA	192.

530	KNSFYLEMNSLRVGDTAVYYC**AREGIRTPYDY**WGQGARVTVSS	
VH 546	EVQLVESGGGLVQPGGSLRLSCAAS**GFTLSSYD**MHWVRQATGKGLEWVSA**IGSTGDT**YYTGSVMGRFTISRDAA KNSFYLEMNSLRVGDTAVYYC**AREGIRTPYDY**WGQGTLVTVSS	193.
VH 550	EVQLVESGGGLVQPGGSLRLSCAAS**GFTLSSYD**MHWVRQATGKGLEWVSA**IGSTGDT**YYPGSVKGRFTISRENA KNSLYLQMNSLRAGDTAVYYC**AREGIRTPYDY**WGQGTLVTVSS	194.
VH 554	EVQLVESGGGLVQPGGSLRLSCAAS**GFTLSSYD**MHWVRQATGKGLEWVSA**IGSTGDT**YYTGSVMGRFTISRDAA KNSFYLEMNSLRVGDTAVYYC**AREGIRTPYDY**WGQGARVTVSS	195.
VH 570	EVQLVESGGGLVQPGGSLRLSCAAS**GFTLSSYD**MHWVRQATGKGLEWVSA**IGSTGDT**YYTGSVMGRFTISRDAA KNSFYLEMNSLRVGDTAVYYC**AREGIRTPYDY**WGQGTLVTVSS	196.
VH 574	EVQLVESGGGLVQPGGSLRLSCAAS**GFTLSSYD**MHWVRQATGKGLEWVSA**IGSTGDT**YYPGSVKGRFTISRENA KNSLYLQMNSLRAGDTAVYYC**AREGIRTPYDY**WGQGTLVTVSS	197.
VH 578	EVQLVESGGGLVQPGRSLRLSCAAS**GFTFDDYA**MHWVRQAPGKGLEWVSG**INWNSGSI**GYADSVKGRFTISRDN AKHSLYLQMNSLRPEDTALYYC**VKEVTTGYYYGMDV**WGQGTTVTVSS	198.
VH 594	EVQLVESGGGLVQPGRSLRLSCAAS**GFTFDDYA**MHWVRQAPGKGLEWVSG**INWNSGSI**GYADSVKGRFTISRDN AKHSLYLQMNSLRPEDTALYYC**VKEVTTGYYYGMDV**WGQGTTVTVSS	199.
VH 598	EVQLVESGGGLVQPGRSLRLSCAAS**GFTFDDYA**MHWVRQAPGKGLEWVSG**INWNSGSI**GYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTALYYC**VKEVTTGYYYGMDV**WGQGTTVTVSS	200.
VH 602	EVQLLESGGGLVQPGGSLRLSCAAS**GFTFSSYA**MNWVRQAPGKGLDWVSG**ISGNGGST**YYADSVKGRFTISRDI SKNTLYVQMHSLRVEDTAVYYC**AKARYYDFWGGNFDL**WGRGTQVTVSS	201.
VH 618	EVQLLESGGGLVQPGGSLRLSCAAS**GFTFSSYA**MNWVRQAPGKGLDWVSG**ISGNGGST**YYADSVKGRFTISRDI SKNTLYVQMHSLRVEDTAVYYC**AKARYYDFWGGNFDL**WGRGTLVTVSS	202.
VH 622	EVQLLESGGGLVQPGGSLRLSCAAS**GFTFSSYA**MSWVRQAPGKGLEWVSA**ISGNGGST**YYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC**AKARYYDFWGGNFDL**WGRGTLVTVSS	203.
VH 626	QVQLVQSGPEVKNPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NDAQKFQDRVAMTTDT STSTAYMELRSLRSDDTAIYYC**SRDRLVVPPALYYSYYVMDV**WGQGTTVTVSS	204.
VH 642	QVQLVQSGPEVKNPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NDAQKFQDRVAMTTDT STSTAYMELRSLRSDDTAIYYC**SRDRLVVPPALYYSYYVMDV**WGQGTTVTVSS	205.
VH 646	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NYAQKLQGRVTMTTDT STSTAYMELRSLRSDDTAVYYC**SRDRLVVPPALYYSYYVMDV**WGQGTTVTVSS	206.
VH 650	QVQLVQSGPEVKNPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NDAQKFQDRVAMTTDT STSTAYMELRSLRSDDTAIYYC**SRDRLVVPPALNYYYYVMDV**WGQGTTVTVSS	207.
VH 666	QVQLVQSGPEVKNPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NDAQKFQDRVAMTTDT STSTAYMELRSLRSDDTAIYYC**SRDRLVVPPALNYYYYVMDV**WGQGTTVTVSS	208.

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VH 670	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NYAQKLQGRVTMTTDT STSTAYMELRSLRSDDTAVYYC**SRDRLVVPPALNYYYYVMDV**WGQGTTVTVSS	209.
VH 674	QVQLVQSGPEVKNPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NDAQKFQDRVAMTTDT STSTAYMELRSLRSDDTAIYYC**SRDRLVVPPALYYYYYVMDV**WGQGTTVTVSS	210.
VH 690	QVQLVQSGPEVKNPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NDAQKFQDRVAMTTDT STSTAYMELRSLRSDDTAIYYC**SRDRLVVPPALYYYYYVMDV**WGQGTTVTVSS	211.
VH 694	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTTYG**ISWVRQAPGQGLEWMGW**ISGYNGKT**NYAQKLQGRVTMTTDT STSTAYMELRSLRSDDTAVYYC**SRDRLVVPPALYYYYYVMDV**WGQGTTVTVSS	212.
VH 698	QVHLVESGGGLVKPGGSLRLSCAAS**GFTFSDHY**MSWIRQAPGKGLEWISY**ISNDGGTK**YYVDSVEGRFIISRDN AKNSLYLHMNSLRADDTAVYYC**ARDQGYIGYDSYYYYSYGMDV**WGQGTTVTVAS	213.
VH 714	QVQLVESGGGLVKPGGSLRLSCAAS**GFTFSDHY**MSWIRQAPGKGLEWISY**ISNDGGTK**YYVDSVEGRFIISRDN AKNSLYLHMNSLRADDTAVYYC**ARDQGYIGYDSYYYYSYGMDV**WGQGTTVTVSS	214.
VH 718	QVQLVESGGGLVKPGGSLRLSCAAS**GFTFSDHY**MSWIRQAPGKGLEWVSY**ISNDGGTK**YYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYC**ARDQGYIGYDSYYYYSYGMDV**WGQGTTVTVSS	215.
VH 722	QILLVQSGPEVKEPGASVKVSCKAS**GYTFTNYA**ISWVRQVPGQGLEWMGW**VSAYNGHT**NYAHEVQGRVTMTTDT STTTAYMELRSLRSDDTAMYYC**ARGGVVVPVAPHFYNGMDV**WGQGTTVTVSS	216.
VH 738	QVQLVQSGPEVKEPGASVKVSCKAS**GYTFTNYA**ISWVRQVPGQGLEWMGW**VSAYNGHT**NYAHEVQGRVTMTTDT STTTAYMELRSLRSDDTAMYYC**ARGGVVVPVAPHFYNGMDV**WGQGTTVTVSS	217.
VH 742	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTNYA**ISWVRQAPGQGLEWMGW**VSAYNGHT**NYAQKLQGRVTMTTDT STSTAYMELRSLRSDDTAVYYC**ARGGVVVPVAPHFYNGMDV**WGQGTTVTVSS	218.
VL 68	DIVMTQSPDSLAVSLGERATINCKSS**QSVFHTSNNKNY**LVWYQQKPGQPPKLLLY**WAS**TRESGVPDRFSGSGSG TDFTLTISSLQAEDVANYYC**HQYYSIPWT**FGQGTKVEIK	219.
VL 92 H1H316P	DIVMTQSPDSLAVSLGERATINCKSS**QSVLYRSNNRNF**LGWYQQKPGQPPNLLIY**WAS**TRESGVPDRFSGSGSG TDFTLTISSLQAEDVAVYYC**QQYYTTPYT**FGQGTKLEIK	220.
VL 140	DIQMTQSPSFLSASVGDRVTITCWAS**QDISSY**LAWYQQKPGIAPKLLIY**AAS**TLQSGVPSRFGGSGSGTEFTLT ISSLQPEDFATYYC**QQLNSYPRT**FGQGTKVEIK	221.
VL 226 H1M300N	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSNGNNY**LDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQTLQTPLT**FGGGTKVEIK	222.
VL 10	DIQMTQSPATLSVSPGERAALSCRAS**QSVSSN**LAWYHQKPGQAPRLLIY**GAS**TRATGIPARFSGIGSGTEFTLI ISSLQSEDFAFYFC**QQYNNWPPFT**FGPGTKVEIKR	223.
VL	EIVMTQSPATLSVSPGERAALSCRAS**QSVSSN**LAWYHQKPGQAPRLLIY**GAS**TRATGIPARFSGIGSGTEFTLI	224.

20	ISSLQSEDFAFYFC**QQYNNWPPFT**FGPGTKVDIK	
VL 24	EIVMTQSPATLSVSPGERATLSCRAS**QSVSSN**LAWYQQKPGQAPRLLIY**GAS**TRATGIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC**QQYNNWPPFT**FGPGTKVDIK	225.
VL 34	AIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQKPGKAPKLLIY**KAS**SLESGVPSRFSGSGSGTEFTLT ISSLQPDDFATYYC**QQYNSYYT**FGQGTKVEIKR	226.
VL 44	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQKPGKAPKLLIY**KAS**SLESGVPSRFSGSGSGTEFTLT ISSLQPDDFATYYC**QQYNSYYT**FGQGTKLEIK	227.
VL 48	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQKPGKAPKLLIY**KAS**SLESGVPSRFSGSGSGTEFTLT ISSLQPDDFATYYC**QQYNSYYT**FGQGTKLEIK	228.
VL 58	AIQMTQSPDSLAVSLGERATINCKSS**QSVFHTSNNKNY**LVWYQQKPGQPPKLLLY**WAS**TRESGVPDRFSGSGSG TDFTLTISSLQAEDVANYYC**HQYYSIPWT**FGQGTKVEIKR	229.
VL 72	DIVMTQSPDSLAVSLGERATINCKSS**QSVFHTSNNKNY**LAWYQQKPGQPPKLLIY**WAS**TRESGVPDRFSGSGSG TDFTLTISSLQAEDVAVYYC**HQYYSIPWT**FGQGTKVEIK	230.
VL 82	DIQMTQSPDSLAVSLGERATINCKSS**QSVLYRSNNRNF**LGWYQQKPGQPPNLLIY**WAS**TRESGVPDRFSGSGSG TDFTLTISSLQAEDVAVYYC**QQYYTTPYT**FGQGTKVEIKR	231.
VL 96	DIVMTQSPDSLAVSLGERATINCKSS**QSVLYRSNNRNF**LAWYQQKPGQPPKLLIY**WAS**TRESGVPDRFSGSGSG TDFTLTISSLQAEDVAVYYC**QQYYTTPYT**FGQGTKLEIK	232.
VL 106	AIQLTQSPSSLSASVGDRVTITCRAS**QDIRND**LGWYQQKPGKAPKLLIY**AAS**SLQSGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYC**LQDYNYPWT**FGQGTKVEIKR	233.
VL 116	AIQMTQSPSSLSASVGDRVTITCRAS**QDIRND**LGWYQQKPGKAPKLLIY**AAS**SLQSGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYC**LQDYNYPWT**FGQGTKVEIK	234.
VL 120	AIQMTQSPSSLSASVGDRVTITCRAS**QDIRND**LGWYQQKPGKAPKLLIY**AAS**SLQSGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYC**LQDYNYPWT**FGQGTKVEIK	235.
VL 130	DIQMTQSPSFLSASVGDRVTITCWAS**QDISSY**LAWYQQKPGIAPKLLIY**AAS**TLQSGVPSRFGGSGSGTEFTLT ISSLQPEDFATYYC**QQLNSYPRT**FGQGTKVEIKR	236.
VL 144	DIQMTQSPSSLSASVGDRVTITCRAS**QDISSY**LGWYQQKPGKAPKRLIY**AAS**SLQSGVPSRFSGSGSGTEFTLT ISSLQPEDFATYYC**QQLNSYPRT**FGQGTKVEIK	237.
VL 154	AIQMTQSPLSLSVTLGQPASISCRSS**QSLVYSDGDTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT AFTLKISGVEAEDVGVYYC**MQATHWPRT**FGQGTKVEIKR	238.
VL 164	DVVMTQSPLSLSVTLGQPASISCRSS**QSLVYSDGDTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT AFTLKISGVEAEDVGVYYC**MQATHWPRT**FGQGTKVEIK	239.
VL 168	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGDTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQATHWPRT**FGQGTKVEIK	240.

VL 178	DIQMTQSPLSLPVTPGEPASISCRSS**QSLLHSHGYDY**LDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPLT**FGGGTKVEIKR	241.
VL 188	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSHGYDY**LDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPLT**FGGGTKVEIK	242.
VL 192	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSHGYDY**LDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPLT**FGGGTKVEIK	243.
VL 202	DIQMTQSPLSLPVTPGEPASISCRSS**QSLLHSHGYNY**LDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGGGSGT DFTLKISRVEAEDVGIYYC**MQALQTPLT**FGGGTKVEIKR	244.
VL 212	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSHGYNY**LDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGGGSGT DFTLKISRVEAEDVGIYYC**MQALQTPLT**FGGGTKVEIK	245.
VL 216	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSHGYNY**LDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPLT**FGGGTKVEIK	246.
VL 236	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSNGNNY**LDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQTLQTPLT**FGGGTKVEIK	247.
VL 240	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSNGNNY**LDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQTLQTPLT**FGGGTKVEIK	248.
VL 250	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSNGYNY**LDWYLQKPGQSPQLLIY**LGF**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPLT**FGGGTKVEIR	249.
VL 260	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSNGYNY**LDWYLQKPGQSPQLLIY**LGF**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPLT**FGGGTKVEIK	250.
VL 264	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSNGYNY**LDWYLQKPGQSPQLLIY**LGF**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPLT**FGGGTKVEIK	251.
VL 274	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSNGYNY**LDWYLQKPGQSPQLLIY**LGF**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPLT**FGGGTKVEIR	252.
VL 284	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSNGYNY**LDWYLQKPGQSPQLLIY**LGF**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPLT**FGGGTKVEIK	253.
VL 288	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHSNGYNY**LDWYLQKPGQSPQLLIY**LGF**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPLT**FGGGTKVEIK	254.
VL 298	DIVMTQSPLSLPVTPGEPASISCRSS**QTLLHSNGYNY**FDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGIYYC**MQALQTPLT**FGGGTKVEIR	255.
VL 308	DIVMTQSPLSLPVTPGEPASISCRSS**QTLLHSNGYNY**FDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGIYYC**MQALQTPLT**FGGGTKVEIK	256.
VL	DIVMTQSPLSLPVTPGEPASISCRSS**QTLLHSNGYNY**LDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGSGSGT	257.

312	DFTLKISRVEAEDVGVYYC**MQALQTPLT**FGGGTKVEIK	
VL 322	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWSQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	258.
VL 332	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWSQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	259.
VL 336	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	260.
VL 346	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQRPGKAPKLLIY**KAS**SLEGGVPSRFSGSGSGTEFTLT ISSLQPEDFATYYC**QQYNSYWYT**FGQGTKLEIK	261.
VL 356	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQRPGKAPKLLIY**KAS**SLEGGVPSRFSGSGSGTEFTLT ISSLQPEDFATYYC**QQYNSYWYT**FGQGTKLEIK	262.
VL 360	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQKPGKAPKLLIY**KAS**SLESGVPSRFSGSGSGTEFTLT ISSLQPDDFATYYC**QQYNSYWYT**FGQGTKLEIK	263.
VL 370	KIVLTQSPGTLPLFPGERATLSCRAS**QSVNNKF**LAWYQQKSGQAPRLLIY**GAS**SRATGIPDRFSGSGSGTDFTL TISGLEPEDFEVYYC**QVYGNSLT**LGGGTKVEIK	264.
VL 380	EIVLTQSPGTLPLFPGERATLSCRAS**QSVNNKF**LAWYQQKSGQAPRLLIY**GAS**SRATGIPDRFSGSGSGTDFTL TISGLEPEDFEVYYC**QVYGNSLT**LGGGTKVEIK	265.
VL 384	EIVLTQSPGTLSLSPGERATLSCRAS**QSVNNKF**LAWYQQKPGQAPRLLIY**GAS**SRATGIPDRFSGSGSGTDFTL TISRLEPEDFAVYYC**QVYGNSLT**FGGGTKVEIK	266.
VL 394	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQIPGKAPKLLIY**KAS**SLENGVPSRFSGSGSGTEFTLI ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	267.
VL 404	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQIPGKAPKLLIY**KAS**SLENGVPSRFSGSGSGTEFTLI ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	268.
VL 408	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQKPGKAPKLLIY**KAS**SLESGVPSRFSGSGSGTEFTLT ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	269.
VL 418	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQIPGKAPKLLIY**KAS**SLENGVPSRFSGSGSGTEFTLI ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	270.
VL 428	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQIPGKAPKLLIY**KAS**SLENGVPSRFSGSGSGTEFTLI ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	271.
VL 432	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQKPGKAPKLLIY**KAS**SLESGVPSRFSGSGSGTEFTLT ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	272.
VL 442	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQVPGKAPKLLIY**KAS**SLENGVPSRFSGSGSGTEFTLI ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	273.

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VL 452	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQVPGKAPKLLIY**KAS**SLENGVPSRFSGSGSGTEFTLI ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	274.
VL 456	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQKPGKAPKLLIY**KAS**SLESGVPSRFSGSGSGTEFTLT ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	275.
VL 466	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQIPGKAPKLLIY**KAS**SLENGVPSRFSGSGSGTEFTLI ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	276.
VL 476	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQIPGKAPKLLIY**KAS**SLENGVPSRFSGSGSGTEFTLI ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	277.
VL 480	DIQMTQSPSTLSASVGDRVTITCRAS**QSISSW**LAWYQQKPGKAPKLLIY**KAS**SLESGVPSRFSGSGSGTEFTLT ISSLQPDDFATYYC**QQYISYSRT**FGQGTKVEIK	278.
VL 490	EIVMTQSPATLSVSPGERGTLSCRAS**QSVSSN**LAWYQQKPGQAPRLLIY**GAS**TRATGFPARFSGSGSGTEFTLT ISSLQSEDFAVYYC**QQYNKWPPFT**FGPGTKVDFK	279.
VL 500	EIVMTQSPATLSVSPGERGTLSCRAS**QSVSSN**LAWYQQKPGQAPRLLIY**GAS**TRATGFPARFSGSGSGTEFTLT ISSLQSEDFAVYYC**QQYNKWPPFT**FGPGTKVDIK	280.
VL 504	EIVMTQSPATLSVSPGERATLSCRAS**QSVSSN**LAWYQQKPGQAPRLLIY**GAS**TRATGIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC**QQYNKWPPFT**FGPGTKVDIK	281.
VL 514	DIVMTQFPLSLPVTPGEPASISCRSS**QSLLHINEYNY**LDWYLKKPGQSPQLLIY**LGF**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPWT**LGQGTKVEIK	282.
VL 524	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHINEYNY**LDWYLKKPGQSPQLLIY**LGF**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPWT**LGQGTKVEIK	283.
VL 528	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHINEYNY**LDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPWT**FGQGTKVEIK	284.
VL 538	EIVMTQSPATLSVSPGERATLSCRAS**QSVSSN**VAWYQQKPGQAPRLLIY**GAS**TRATGIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC**QQYNNWPPFT**FGPGTKVDIK	285.
VL 548	EIVMTQSPATLSVSPGERATLSCRAS**QSVSSN**VAWYQQKPGQAPRLLIY**GAS**TRATGIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC**QQYNNWPPFT**FGPGTKVDIK	286.
VL 552	EIVMTQSPATLSVSPGERATLSCRAS**QSVSSN**LAWYQQKPGQAPRLLIY**GAS**TRATGIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC**QQYNNWPPFT**FGPGTKVDIK	287.
VL 562	EIVMTQSPATLSVSPGERATLSCRAS**QSVSSN**VAWYQQKPGQAPRLLIY**GAS**TRATGIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC**QQYNNWPPFT**FGPGTKVDIK	288.
VL 572	EIVMTQSPATLSVSPGERATLSCRAS**QSVSSN**VAWYQQKPGQAPRLLIY**GAS**TRATGIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC**QQYNNWPPFT**FGPGTKVDIK	289.
VL	EIVMTQSPATLSVSPGERATLSCRAS**QSVSSN**LAWYQQKPGQAPRLLIY**GAS**TRATGIPARFSGSGSGTEFTLT	290.

576	ISSLQSEDFAVYYC**QQYNNWPPFT**FGPGTKVDIK	
VL 586	DIQLTQSPSFLSASVGDRVTITCWAS**QGISSY**LAWYQKKPGKAPNLLIY**DAS**TLQSGVPSRFSGSGSGTEFTLT LSSLQPEDFATYYC**QQLNIYPFT**FGPGTKVDIK	291.
VL 596	DIQLTQSPSFLSASVGDRVTITCWAS**QGISSY**LAWYQKKPGKAPNLLIY**DAS**TLQSGVPSRFSGSGSGTEFTLT LSSLQPEDFATYYC**QQLNIYPFT**FGPGTKVDIK	292.
VL 600	DIQLTQSPSFLSASVGDRVTITCRAS**QGISSY**LAWYQQKPGKAPKLLIY**DAS**TLQSGVPSRFSGSGSGTEFTLT ISSLQPEDFATYYC**QQLNIYPFT**FGPGTKVDIK	293.
VL 610	EIVLTQSPGTLSLSPGERATLSCRAS**QSVSIRY**LAWYQQKPGQAPRLLIY**GAS**SRATGIPDRFSVSVSGTDFTL TITRLEPEDFAVYYC**QQYGSSPLT**FGGGTKVEIK	294.
VL 620	EIVLTQSPGTLSLSPGERATLSCRAS**QSVSIRY**LAWYQQKPGQAPRLLIY**GAS**SRATGIPDRFSVSVSGTDFTL TITRLEPEDFAVYYC**QQYGSSPLT**FGGGTKVEIK	295.
VL 624	EIVLTQSPGTLSLSPGERATLSCRAS**QSVSIRY**LAWYQQKPGQAPRLLIY**GAS**SRATGIPDRFSGSGSGTDFTL TISRLEPEDFAVYYC**QQYGSSPLT**FGGGTKVEIK	296.
VL 634	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	297.
VL 644	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	298.
VL 648	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	299.
VL 658	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	300.
VL 668	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	301.
VL 672	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	302.
VL 682	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	303.
VL 692	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	304.
VL 696	DVVMTQSPLSLPVTLGQPASISCRSS**QSLVYSDGNTY**LNWFQQRPGQSPRRLIY**KVS**NRDSGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQGTHWPYT**FGQGTKLEIK	305.
VL 706	KIVLTQSPGTLPLFPGERATLSCRAS**QSVNNKF**LAWYQQKSGQAPRLLIY**GAS**SRATGIPDRFSGSGSGTDFTL TISGLEPEDFEVYYC**QVYGNSLT**FGGGTKVEIK	306.

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VL 716	EIVLTQSPGTLPLFPGERATLSCRAS**QSVNNKF**LAWYQQKSGQAPRLLIY**GAS**SRATGIPDRFSGSGSGTDFTL TISGLEPEDFEVYYC**QVYGNSLT**FGGGTKVEIK	307.
VL 720	EIVLTQSPGTLSLSPGERATLSCRAS**QSVNNKF**LAWYQQKPGQAPRLLIY**GAS**SRATGIPDRFSGSGSGTDFTL TISRLEPEDFAVYYC**QVYGNSLT**FGGGTKVEIK	308.
VL 730	DIVMTQFPLSLPVTPGEPASISCRSS**QSLLHINEYNY**LDWYLKKPGQSPQLLIY**LGF**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPWT**FGQGTKVEIK	309.
VL 740	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHINEYNY**LDWYLKKPGQSPQLLIY**LGF**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPWT**FGQGTKVEIK	310.
VL 744	DIVMTQSPLSLPVTPGEPASISCRSS**QSLLHINEYNY**LDWYLQKPGQSPQLLIY**LGS**NRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**MQALQTPWT**FGQGTKVEIK	311.

GROUP E		
VH 508.20	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFTGYAIH**WVRQAPGKGLEWVG**RISPANGNTNYADSVKG**RFTISADT SKNTAYLQMNSLRAEDTAVYYCAR**WIGSRELYIMDY**WGQGTLVTVSS	312.
VH 508.04	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFTGYAIH**WVRQAPGKGLEWVG**RISPANGNTNYADSVKG**RFTISADT SKNTAYLQMNSLRAEDTAVYYCAR**WIGSRELYIMDY**WGQGTLVTVSS	313.
VH 508.06	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFTGYAIH**WVRQAPGKGLEWVG**RISPANGNTNYADSVKG**RFTISADT SKNTAYLQMNSLRAEDTAVYYCAR**WIGSRELYIMDY**WGQGTLVTVSS	314.
VH 508.28	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFTRHTIH**WVRQAPGKGLEWVG**RISPANGNTNYADSVKG**RFTISADT SKNTAYLQMNSLRAEDTAVYYCAR**WIGSRELYIMDY**WGQGTLVTVSS	315.
VH 508.33	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFSSTAIH**WVRQAPGKGLEWVG**RISPANGNTNYADSVKG**RFTISADT SKNTAYLQMNSLRAEDTAVYYCAR**WIGSRELYIMDY**WGQGTLVTVSS	316.
VH 508.84	EVQLVESGGGLVQPGGSLRLSCAAS**GFTFTGYAIH**WVRQAPGKGLEWVG**RISPANGNTNYADSVKG**RFTISADT SKNTAYLQMNSLRAEDTAVYYCAR**WIGSRELYIMDY**WGQGTLVTVSS	317.
VL 508.20	DIQMTQSPSSLSASVGDRVTITC**RASQDVSSAVA**WYQQKPGKAPKLLIY**SASSLYS**GVPSRFSGSRSGTDFTLT ISSLQPEDFATYYC**QQSYTTPPT**FGQGTKVEIKR	318.
VL 508.04	DIQMTQSPSSLSASVGDRVTITC**RASQDVSSAVA**WYQQKPGKAPKLLIY**SASSLYS**GVPSRFSGSRSGTDFTLT ISSLQPEDFATYYC**QQSYPAPAT**FGQGTKVEIKR	319.
VL 508.06	DIQMTQSPSSLSASVGDRVTITC**RASQDVSSAVA**WYQQKPGKAPKLLIY**SASSLYS**GVPSRFSGSRSGTDFTLT ISSLQPEDFATYYC**QQSYPSPAT**FGQGTKVEIKR	320.
VL 508.28	DIQMTQSPSSLSASVGDRVTITC**RASQDVSSAVA**WYQQKPGKAPKLLIY**SASSLYS**GVPSRFSGSRSGTDFTLT ISSLQPEDFATYYC**QQSYRIQPT**FGQGTKVEIKR	321.
VL	DIQMTQSPSSLSASVGDRVTITC**RASQDVSSAVA**WYQQKPGKAPKLLIY**SASSLYS**GVPSRFSGSRSGTDFTLT	322.

508.33	ISSLQPEDFATYYC**QQSYPALHT**FGQGTKVEIKR	
VL 508.84	DIQMTQSPSSLSASVGDRVTITC**RASQDVSSAVA**WYQQKPGKAPKLLIY**SASSLYS**GVPSRFSGSRSGTDFTLT ISSLQPEDFATYYC**QQSYPAPST**FGQGTKVEIKR	323.

GROUP F		
VH	EVQLVESGGGLVKPGGSLRLSCAAS**GFPFSKLGMV**WVRQAPGKGLEWVS**TISSGGGYTYYPDSVKG**RFTISRDN AKNSLYLQMNSLRAEDTAVYYCAR**EGISFQGGTYTYVMDY**WGQGTLVTVSS	324.
VL	DIVMTQSPLSLPVTPGEPASISC**RSSKSLLHRNGITYSY**WYLQKPGQSPQLLIY**QLSNLAS**GVPDRFSGSGSGT DFTLKISRVEAEDVGVYYC**YQNLELPLT**FGQGTKVEIK	325.

GROUP G		
VH IB20	QVQLVQSGAEVKKPGESLKISCKGS**GYSFTNYWIS**WVRQMPGKGLE**WMGIIYPGDSYTNYSPSFQG**QVTISADK SISTAYLQWSSLKASDTAMYYCAR**DYWYKPLFDI**WGQGTLVTVSS	326.
VL IB20	DIVMTQSPDSLAVSLGERATINC**RSSQSVLYSSNNKNYL**AWYQQKPGQPPK**LLIYWASTRES**GVPDRFSGSGSG TDFTLTISSLQAEDVAVYYC**QQYSSFPI**TFGQGTKVEIKR	327.
IB20 Variant HC CDR1	$GYSFTX_1YX_2IX_3$ X_1=N or D X_2=W or Y	328.
IB20 Variant HC CDR2	$WMGX_1IYPGDSX_2TX_3YX_4X_5X_6FQG$ X_1 = I, R, W, L, or M X_2 = Y or D X_3 = N, R, H, S, K, Q X_4 = S or N X_5 = P, Q, H X_6 = S, K, N, or R	329.
IB20 Variant HC CDR3	$DX_1X_2X_3X_4X_5X_6X_7DX_8$ X_1 = Y, R, or H X_2 = W, Y, G, A, or F X_3 = Y or S X_4 = K, R, T, G, S, D, E, H, or N	330.

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	X_5 = P, S, G, D, A, H, R, or Y X_6 = L, Y, F, A, D, H, P, or S X_7 = F or S X_8 = I, V, Y, F, or N	
IB30 Variant VH CDR2	WMGX_1IYPGDSYTX_2YSX_3SFQG X_1 = I or R X_2 = N, R, H, or S X_3 = P or Q	331.
IB30 Variant VH CDR3	DYWY$X_1X_2X_3$FDX_4 X_1 = K, G, R, S, D, E, H, N, or Q X_2 = P, G, D, S, A, H, R, or Y X_3 = L, A, Y, D, F, H, P, S, or V X_4 = I, Y, F, or N	332.
IB20 Variant LC CDR1	X_1 Ser Ser Gln Ser Val X_2 X_3 Ser X_4 X_5 X_6 Lys Asn X_7 Leu X_8 X_1 = Arg, Lys, His, Asn, Gln or Ser X_2 = Leu or Phe X_3 = Tyr or His X_4 = Ser, Arg or Gly X_5 = Asn or Thr X_6 = Asn, Arg, His or Ser X_7 = Tyr or Phe X_8 = Ala or Thr	333.
IB20 Variant LC CDR2	LLIYX_1X_2SX_3RX_4X_5 X_1 = Trp, Phe or Leu X_2 = Ala or Thr X_3 = Thr, Ile, Ala or Val X_4 = Glu, Ala or Lys X_5 = Ser or Thr	334.
IB20 Variant LC CDR3	QQY$X_1X_2X_3$PX_4	335.

	X_1 = Ser or Tyr X_2 = Ser or Thr X_3 = Phe, Tyr, Leu, Thr, His, Ile, Asn, Pro or Ser X_4 = Ile, Arg, Val, Tyr, Asp, Phe, Gly, His, Leu, Asn or Ser	
1B20 Antibody Variant VL CDR1 Sequence	RSSQSVLYSSNNKNX$_1$LX$_2$ X_1 = Tyr or Phe X_2 = Ala or Thr	336.
1B20 Antibody Variant VL CDR2 Sequence	LLIYX$_1$ASTRX$_2$X$_3$ X_1 = Trp, Phe or Leu X_2 = Glu or Lys X_3 = Ser or Thr	337.
1B20 Antibody Variant VL CDR3 Sequence	QQYSSX$_1$PX$_2$ X_1 = Phe, Tyr, Thr, Ile, Asn or Ser X_2 = Ile, Tyr, Arg, Phe, His, Leu, Asn or Ser	338.
VH; 1B20 VARIANT SEQUENCE	QVQLVQSGAEVKKPGESLKISCKGSGYSFTX$_1$YX$_2$IX$_3$WVRQMPGKGLEWMGX$_4$IYPGDSX$_5$TX$_6$YX$_7$X$_8$X$_9$F QGQVTISADKSISTAYLQWSSLKASDTAMYYCARDX$_{10}$X$_{11}$X$_{12}$X$_{13}$X$_{14}$X$_{15}$X$_{16}$DX$_{17}$WGQGTLVTVSS X_1 = Asn or Asp X_2 = Trp or Tyr X_3 = Ser, Thr or Ala X_4 = Ile, Arg, Trp, Leu or Met X_5 = Tyr or Asp X_6 = Asn, Arg, His, Ser, Lys or Gln X_7 = Ser or Asn X_8 = Pro, Gln or His X_9 = Ser, Lys, Asn or Arg X_{10} = Tyr, Arg or His X_{11} = Trp, Tyr or Gly X_{12} = Tyr or Ser	339.

	X_{13} = Lys, Arg, Thr, Gly, Ser, Asp, Glu, His, Asn or Gln X_{14} = Pro, Ser, Gly, Asp, Ala, His, Arg or Tyr X_{15} = Leu, Tyr, Phe, Ala, Asp, His, Pro, Ser or Val X_{16} = Phe or Ser X_{17} = Ile, Val, Tyr, Phe or Asn	
VH; 1B20 VARIANT SEQUENCE	QVQLVQSGAEVKKPGESLKISCKGSGYSFTX$_1$YX$_2$IX$_3$WVRQMPGKGLEWMGX$_4$IYPGDSYTX$_5$YSX$_6$SFQGQ VTISADKSISTAYLQWSSLKASDTAMYYCARDWYWX$_7$X$_8$X$_9$FDX$_{10}$WGQGTLVTVSS X_1 = Asn or Asp X_2 = Trp or Tyr X_3 = Ser, Thr or Ala X_4 = Ile or Arg X_5 = Asn, Arg, His, Ser, Lys or Gln X_6 = Pro or Gln X_7 = Lys, Gly, Arg, Ser, Asp, Glu, His, Asn or Gln X_8 = Pro, Gly, Asp, Ser, Ala, His, Arg or Tyr X_9 = Leu, Ala, Tyr, Asp, Phe, His, Pro, Ser or Val X_{10} = Ile, Tyr, Phe or Asn	340.
VL; 1B20 Variant Sequence	DIVMTQSPDSLAVSLGERATINCX$_1$SSQSVX$_2$X$_3$SX$_4$X$_5$X$_6$KNX$_7$LX$_8$WYQQKPGQPPKLLIYX$_9$X$_{10}$SX$_{11}$RX$_{12}$ X$_{13}$GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYX$_{14}$X$_{15}$X$_{16}$PX$_{17}$TFGQGTKVEIKR X_1 = Arg, Lys, His, Asn, Gln or Ser X_2 = Leu or Phe X_3 = Tyr or His X_4 = Ser, Arg or Gly X_5 = Asn or Thr X_6 = Asn, Arg, His or Ser X_7 = Tyr or Phe X_8 = Ala or Thr X_9 = Trp, Phe or Leu X_{10} = Ala or Thr X_{11} = Thr, Ile, Ala or Val X_{12} = Glu, Ala or Lys X_{13} = Ser or Thr	341.

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	X_{14} = Ser or Tyr X_{15} = Ser or Thr X_{16} = Phe, Tyr, Leu, Thr, His, Ile, Asn, Pro or Ser X_{17} = Ile, Arg, Val, Tyr, Asp, Phe, Gly, His, Leu, Asn or Ser	
VL; 1B20 VARIANT SEQUENCE	DIVMTQSPDSLAVSLGERATINCRSSQSVLYSSNNKNX_1LX_2WYQQKPGQPPKLLIYX_3ASTR$X_4$$X_5$GVPDRFSGS GSGTDFTLTISSLQAEDVAVYYCQQYSSX_6PX_7TFGQGTKVEIKR X_1 = Y or F X_2 = A or T X_3 = W, F, or L X_4 = E or K X_5 = S or T X_6 = F, Y, T, I, N, or S X_7 = I, Y, R, F, H, L, N, or S	342.
VH; 1B20 Variant Sequence F120	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGLIYPGDSYTRY NPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYYSRPFSDIWGQGTLVTVSS	343.
VH; 1B20 Variant Sequence F116	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGLIYPGDSYTRY NPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYYSRPYSDVWGQGTLVTVSS	344.
VH; 1B20 Variant Sequence F119	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGLIYPGDSYTRY SPNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHGYRPYSDIWGQGTLVTVSS	345.
VH; 1B20 Variant Sequence F113	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGLIYPGDSYTNY NPNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHYSTPFFDVWGQGTLVTVSS	346.
VH; 1B20 Variant Sequence E2	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGLIYPGDSYTRY NRKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYYSRPLSDVWGQGTLVTVSS	347.

VH; 1B20 Variant Sequence G4	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGLIYPGDSYTRY SPRFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHGYKPYSDIWGQGTLVTVSS	348.
VH; 1B20 Variant Sequence F4	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGLIYPGDSYTRY SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYYSKPLFDVWGQGTLVTVSS	349.
VH; 1B20 Variant Sequence B9	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGIIYPGDSYIHY NQNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYYSRPFSDIWGQGTLVTVSS	350.
VH; 1B20 Variant Sequence C3	QVKLVQSGAEVKKPGESLKISCKGSGYSFTNYYIAWVRQMPGKGLEWMGIIYPGDSYTHY NPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHGYKPFSDIWGQGTLVTVSS	351.
VH; 1B20 Variant Sequence F2	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYIAWVRQMPGKGLEWMGVIYPGDSYTRY NPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSRPYFDIWGQGTLVTVSS	352.
VH; 1B20 Variant Sequence F7	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYIAWVRQMPGKGLEWMGIIYPGDSYTHY NPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWSRPFSDVWGQGTLVTVSS	353.
VH; 1B20 Variant Sequence A7	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGIIYPGDSYTRY NPNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSKPLSDVWGQGTLVTVSS	354.
VH; 1B20 Variant Sequence G8	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGLIYPGDSYTHY NPNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWSKPYFDVWGQGTLVTVSS	355.
VH; 1B20 Variant Sequence H4	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYITWVRQMPGRGLEWMGIIYPGDSYTRY NPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWSKPFSDVWGQGTLVTVSS	356.

VH; 1B20 Variant Sequence D5	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYITWVRQMPGKGLEWMGLIYPGDSYTRY SPRFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWSRPYSDVWGQGTLVTVSS	357.
VH; 1B20 Variant Sequence D4	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYIAWVRQMPGKGLEWMGIIYPGDSYTRY NPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSRPFSDVWGQGTLVTVSS	358.
VH; 1B20 Variant Sequence B4	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGLIYPGDSYTHY NPNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSKPLFDVWGQGTLVTVSS	359.
VH; 1B20 Variant Sequence H1	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYIAWVRQMPGKGLEWMGIIYPGDSYTRY NPRFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSKPLSDIWGQGTLVTVSS	360.
VH; 1B20 Variant Sequence G2	QVKLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGIIYPGDSYTHY NPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDRWSKPLFDVWGQGTLVTVSS	361.
VH; 1B20 Variant Sequence A1	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGLIYPGDSYTRY NPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSKPLSDIWGQGTLVTVSS	362.
VH; 1B20 Variant Sequence A4	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGSIYPGDSYTHY SHKFQGQVTISADKSISTAYLQWSSLKASDTAIYYCARDHWSRPFFDVWGQGTLVTVSS	363.
VH; 1B20 Variant Sequence C2	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYIAWVRQMPGKGLEWMGLIYPGDSYTSY NPRFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSTPYFDIWGQGTLVTVSS	364.
VH; 1B20 Variant Sequence H5	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGIIYPGDSYTSY SPRFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSRPLFDVWGQGTLVTVSS	365.
VH; 1B20 Variant Sequence F6	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGLIYPGDSYTHY NPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWYKPFSDIWGQGTLVTVSS	366.
VH; 1B20	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGIIYPGDSYTNY	367.

Variant Sequence B6	NPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWYRPFSDIWGQGTLVTVSS	
VH; 1B20 Variant Sequence B1	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGIIYPGDSYTHY SPNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWYTPFFDVWGQGTLVTVSS	368.
VH; 1B20 Variant Sequence F1	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGLIYPGDSYTNY SPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWSKPLSDVWGQGTLVTVSS	369.
VH; 1B20 Variant Sequence A8	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGMIYPGDSYTHY SPNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSKPYFDVWGQGTLVTVSS	370.
VH; 1B20 Variant Sequence B3	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGMIYPGDSYTSY NPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWSKPYFDIWGQGTLVTVSS	371.
VH; 1B20 Variant Sequence F8	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGMIYPGDSYTNY NQKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSRPYSDIWGQGTLVTVSS	372.
VH; 1B20 Variant Sequence H8	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGMIYPGDSYTHY SQNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWSRPFFDVWGQGTLVTVSS	373.
VH; 1B20 Variant Sequence B5	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGMIYPGDSYTRY SPSFQGQVTISADKSISTAYLQSSSLKASDTAMYYCARDYWYKPFSDVWGQGTLVTVSS	374.
VH; 1B20 Variant Sequence E1	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGMIYPGDSYTSY NPNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWSKPFFDIWGQGTLVTVSS	375.
VH; 1B20 Variant	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYITWVRQMPGKGLEWMGMIYPGDSYTRY SPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWSKPLSDVWGQGTLVTVSS	376.

Sequence E8		
VH; 1B20 Variant Sequence C1	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGMIYPGDSYTRY SPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWYRPYSDIWGQGTLVTVSS	377.
VH; 1B20 Variant Sequence H3	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYITWVRQMPGKGLEWMGMIYPGDSYTHY SQRFQGQVTISADKSISTAYLQWSSLKASDTAIYYCARDHWSRPLFDVWGQGTLVTVSS	378.
VH; 1B20 Variant Sequence A9	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGMIYPGDSYTRY SPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWYRPYSDIWGQGTLVTVSS	379.
VH; 1B20 Variant Sequence G7	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGWIYPGDSYTHY SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWSKPYFDVWGQGTLVTVSS	380.
VH; 1B20 Variant Sequence C6	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYITWVRQMPGKGLEWMGMIYPGDSYTHY NPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWYKPLFDIWGQGTLVTVSS	381.
VH; 1B20 Variant Sequence G6	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYINWVRQMPGKGLEWMGMIYPGDSYTNY NPKFQGQVTISADKSISTAYLQWSSLKASDTAIYYCARDHWSRPFSDVWGQGTLVTVSS	382.
VH; 1B20 Variant Sequence E4	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGWIYPGDSYTSY NQKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSKPYSDVWGQGTLVTVSS	383.
VH; 1B20 Variant Sequence F5	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYITWVRQMPGKGLEWMGMIYPGDSYTNY RHNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSRPYFDIWGQGTLVTVSS	384.
VH; 1B20 Variant Sequence C7	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGWIYPGDSYTHY SHNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWSRPFSDVWGQGTLVTVSS	385.
VH; 1B20 Variant	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGMIYPGDSYTSY SPRFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWYRPFSDIWGQGTLVTVSS	386.

Sequence E3		
VH; 1B20 Variant Sequence D3	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYITWVRQMPGKGLEWMGMIYPGDSYTRY SPKFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSKPFFDVWGQGTLVTVSS	387.
VH; 1B20 Variant Sequence D8	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGMIYPGDSYTHY SQSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWYRPLFDIWGQGTLVTVSS	388.
VH; 1B20 Variant Sequence C8	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYIAWVRQMPGKGLEWMGMIYPGDSYTSY SHRFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWYRPYFDIWGQGTLVTVSS	389.
VH; 1B20 Variant Sequence E5	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGWIYPGDSYTHY NPNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDRWSTPYFDVWGQGTLVTVSS	390.
VH; 1B20 Variant Sequence B8	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYYISWVRQMPGKGLEWMGMIYPGDSYTHY SPNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWSKPYFDVWGQGTLVTVSS	391.
VH; 1B20 Variant Sequence H7	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGPEWMGMIYPGDSYTRY SQKFQGQVTISADKSISTAYLQWSSLKASDTAIYYCARDHWSRPLSDIWGQGTLVTVSS	392.
VH; 1B20 Variant Sequence A5	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGWIYPGDSYTHY NPMFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDRWSRPYFDIWGQGTLVTVSS	393.
VH; 1B20 Variant Sequence A3	QVQLVQSGAEVKKPGESLKISCKGSGYSFTDYYISWVRQMPGKGLEWMGMIYPGDSYTRY SPNFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDHWYRPLSDIWGQGTLVTVSS	394.
1B20 Antibody Variant VH Sequence N59K	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYWISWVRQMPGKGLEWMGIIYPGDSYTKY SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWYKPLFDIWGQGTLVTVSS	395.
1B20 Antibody	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYWISWVRQMPGKGLEWMGIIYPGDSYTQY SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWYKPLFDIWGQGTLVTVSS	396.

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Variant VH Sequence N59Q		
1B20 Antibody Variant VH Sequence N59R	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYWISWVRQMPGKGLEWMGIIYPGDSYTRY SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYWYKPLFDIWGQGTLVTVSS	397.
1B20 Antibody Variant VH Sequence W101A	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYWISWVRQMPGKGLEWMGIIYPGDSYTNY SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYAYKPLFDIWGQGTLVTVSS	398.
1B20 Antibody Variant VH Sequence W101F	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYWISWVRQMPGKGLEWMGIIYPGDSYTNY SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYFYKPLFDIWGQGTLVTVSS	399.
1B20 Antibody Variant VH Sequence W101Y	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYWISWVRQMPGKGLEWMGIIYPGDSYTNY SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARDYYYKPLFDIWGQGTLVTVSS	400.

GROUP H		
Linker Sequence (used in DSB#1)	GGGGGGGGGGGCGG	401.
Linker Seqeunce	GGGGGGGGGGGGCG	402.

(used in DSB#2)		
Linker	GGGGSGGGGS	403.

Group I		
PC9#3 variable heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFNNYAMNWVRQAPGKGLDWVSTISGSGGTTNYADSVKGRFIISRDS SKHTLYLQMNSLRAEDTAVYYCAKDSNWGNFDLWGRGTLVTVSS	404.
PC9#3 variable light chain	DIVMTQSPDSLAVSLGERATINCKSSQSVLYRSNNRNFLGWYQQKPGQPPNLLIYWASTRESGVPDRFSGSGSG TDFTLTISSLQAEDVAVYYCQQYYTTPYTFGQGTKLEIK	405.
PC9#4 variable heavy chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSSYISYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYFCARDYDFWSAYYDAFDVWGQGTMVTVSS	406.
PC9#4 variable light chain	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLISGNSNRPSGVPDRFSGSKSGTSAS LAITGLQAEDEADYYCQSYDSSLSGSVFGGGTKLTVL	407.
PC9#5 variable heavy chain	EMQLVESGGGLVQPGGSLRLSCAASGFTFSSHWMKWVRQAPGKGLEWVANINQDGSEKYYVDSVKGRFTISRDN AKNSLFLQMNSLRAEDTAVYYCARDIVLMVYDMDYYYYGMDVWGQGTTVTVSS	408.
PC9#5 variable light chain	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGNNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYCMQTLQTPLTFGGGTKVEIK	409.
PC9#6 variable heavy chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFNSHAISWVRQAPGQGLEWMGGINPILGIANYAQKFQGRVTITADE STSTAYMELSSLRSEDTAVYYCARHYEIQIGRYGMNVYYLMYRFASWGQGTLVTVSS	410.
PC9#6 variable light chain	DIQMTQSPSSLSASVGDRVTITCRASQGIRSALNWYQQKPGKAPKLLIYNGSTLQSGVPSRFSGSGSGTDFTLT ISSLQPEDFAVYYCQQFDGDPTFGQGTKVEIK	411.
PC9#7 variable heavy chain	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYWISWVRQMPGKGLEWMGIIYPGDSYTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARDYWYKPLFDIWGQGTLVTVSS	412.
PC9#7 variable light chain	DIVMTQSPDSLAVSLGERATINCRSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSG TDFTLTISSLQAEDVAVYYCQQYSSFPITFGQGTKVEIK	413.
NIP228	QVNLRESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGEGLEWVSAISGSGGSTYYADSVKGRFTISRDN	414.

variable heavy chain	SKNTLYLQMNSLRAEDTAVYYCAKRFGEFAFDIWGRGTTVTVSS	
NIP228 variable light chain	AIRMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIK	415.
Human IgG4 Fc fragment	ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKS LSLSLG	416.
Human germline 1-46 (DP-7)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDT STSTVYMELSSLRSEDTAVYYCARD	417.
Human germline VK1 O18 O8 (DPK1)	DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFT ISSLQPEDIATYYCQQ	418.
HS9_DSB7_ V19A_L2	HGEGTFTSDLSKQMEEECARLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSADIQMTQSPSSLSASVGDRVTIT CQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWR TFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	419.
HS9_DSB7_ V19A_L1	HGEGTFTSDLSKQMEEECARLFIEWLKNGGPSSGAPPPGCGGGGGSADIQMTQSPSSLSASVGDRVTITCQASQ DVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRTFGQG TKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	420.
HS9_DSB7_ V19A_L0	HGEGTFTSDLSKQMEEECARLFIEWLKNGGPSSGAPPPGCGADIQMTQSPSSLSASVGDRVTITCQASQDVKTA VAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRTFGQGTKLEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	421.
2.7A4_VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSTYSMNWVRQAPGKGLEWVSSISSSGDYIYYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYCARDLVTSMVAFDYWGQGTLVTVSS	422.
2.7A4_VL	SYELTQPPSVSVSPGQTARITCSGDALPQKYVFWYQQKSGQAPVLVIYEDSKRPSGIPERFSGSSSGTMATLTI SGAQVEDEADYYCYSTDRSGNHRVFGGGTKLTVL	423.

GROUP J		
HS1_VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEI**K**PSGGSTSYNQKFQGRVTMTRDTSTST VYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSS	424.
HS1_VL	DIQMTQSPSSLSASVGDRVTITCQASQDV**Y**TAVAWYQQKPGKAPKLLIY**Y**ASYRYTGVPSRFSGSGSGTDFTFTISSL QPEDIATYYCQQRYSLWRTFGQGTKLEIK	425.
HS2_VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEI**K**PSGGSTSYNQKFQGRVTMTRDTSTST VYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSS	426.
HS2_VL	DIQMTQSPSSLSASVGDRVTITCQASQDV**Y**TAVAWYQQKPGKAPKLLIY**S**ASYRYTGVPSRFSGSGSGTDFTFTISSL QPEDIATYYCQQRYSLWRTFGQGTKLEIK	427.
HS3_VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEI**K**PSGGSTSYNQKFQGRVTMTRDTSTST VYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSS	428.
HS3_VL	DIQMTQSPSSLSASVGDRVTITCQASQDV**K**TAVAWYQQKPGKAPKLLIY**Y**ASYRYTGVPSRFSGSGSGTDFTFTISSL QPEDIATYYCQQRYSLWRTFGQGTKLEIK	429.
HS4_VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEI**K**PSGGSTSYNQKFQGRVTMTRDTSTST VYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSS	430.
HS4_VL	DIQMTQSPSSLSASVGDRVTITCQASQDV**K**TAVAWYQQKPGKAPKLLIY**S**ASYRYTGVPSRFSGSGSGTDFTFTISSL QPEDIATYYCQQRYSLWRTFGQGTKLEIK	431.
HS5_V	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEI**K**PSGGSTSYNQKFQGRVTMTRDTSTST	432.

H	VYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSS	
HS5_VL	DIQMTQSPSSLSASVGDRVTITCQASQDV**S**TAVAWYQQKPGKAPKLLIY**S**ASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRTFGQGTKLEIK	433.
HS6_VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEI**S**PSGGSTSYNQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSS	434.
HS6_VL	DIQMTQSPSSLSASVGDRVTITCQASQDV**Y**TAVAWYQQKPGKAPKLLIY**Y**ASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRTFGQGTKLEIK	435.
HS7_VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEI**S**PSGGSTSYNQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSS	436.
HS7_VL	DIQMTQSPSSLSASVGDRVTITCQASQDV**Y**TAVAWYQQKPGKAPKLLIY**S**ASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRTFGQGTKLEIK	437.
HS8_VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEI**S**PSGGSTSYNQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSS	438.
HS8_VL	DIQMTQSPSSLSASVGDRVTITCQASQDV**K**TAVAWYQQKPGKAPKLLIY**Y**ASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRTFGQGTKLEIK	439.
HS9_VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEI**S**PSGGSTSYNQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSS	440.
HS9_VL	DIQMTQSPSSLSASVGDRVTITCQASQDV**K**TAVAWYQQKPGKAPKLLIY**S**ASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRTFGQGTKLEIK	441.

HS10_VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEI**S**PSGGSTSYNQKFQGRVTMTRDTSTST VYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSS	442.
HS10_VL	DIQMTQSPSSLSASVGDRVTITCQASQDVSTAVAWYQQKPGKAPKLLIY**S**ASYRYTGVPSRFSGSGSGTDFTFTISSL QPEDIATYYCQQRYSLWRTFGQGTKLEIK	443.

HS9_DSB7_G2Y	H**Y**EGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	444.
HS9_DSB7_G2V	H**V**EGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	445.
HS9_DSB7_G2T	H**T**EGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	446.
HS9_DSB7_G2Q	H**Q**EGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	447.
HS9_DSB7_G2N	H**N**EGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	448.
HS9_DSB7_G2I	H**I**EGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	449.
HS9_DSB7_G2F	H**F**EGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	450.
HS9_DSB7_E15G	HGEGTFTSDLSKQM**G**EECVRLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	451.
HS9_DSB7_E15A	HGEGTFTSDLSKQM**A**EECVRLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	452.

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HS9_DS B7_V19T	HGEGTFTSDLSKQMEEEC**T**RLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	453.
HS9_DS B7_V19S	HGEGTFTSDLSKQMEEEC**S**RLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	454.
HS9_DS B7_V19 G	HGEGTFTSDLSKQMEEEC**G**RLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	455.
HS9_DS B7_V19A	HGEGTFTSDLSKQMEEEC**A**RLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	456.
HS9_DS B7_I23T	HGEGTFTSDLSKQMEEECVRLF**T**EWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	457.
HS9_DS B7_I23S	HGEGTFTSDLSKQMEEECVRLF**S**EWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	458.
HS9_DS B7_I23G	HGEGTFTSDLSKQMEEECVRLF**G**EWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	459.
HS9_DS B7_I23A	HGEGTFTSDLSKQMEEECVRLF**A**EWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	460.
HS9_DS B7_L26T	HGEGTFTSDLSKQMEEECVRLFIEW**T**KNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	461.

HS9_DS B7_L26S	HGEGTFTSDLSKQMEEECVRLFIEW**S**KNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	462.
HS9_DS B7_L26P	HGEGTFTSDLSKQMEEECVRLFIEW**P**KNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT	463.

	FGQGTKLEIK	
HS9_DS B7_L26N	HGEGTFTSDLSKQMEEECVRLFIEW**N**KNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	464.
HS9_DS B7_L26Q	HGEGTFTSDLSKQMEEECVRLFIEW**Q**KNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	465.
HS9_DS B7_L26M	HGEGTFTSDLSKQMEEECVRLFIEW**M**KNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	466.
HS9_DS B7_L26I	HGEGTFTSDLSKQMEEECVRLFIEW**I**KNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	467.
HS9_DS B7_L26H	HGEGTFTSDLSKQMEEECVRLFIEW**H**KNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	468.
HS9_DS B7_L26G	HGEGTFTSDLSKQMEEECVRLFIEW**G**KNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	469.
HS9_DS B7_L26E	HGEGTFTSDLSKQMEEECVRLFIEW**E**KNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	470.
HS9_DS B7_L26D	HGEGTFTSDLSKQMEEECVRLFIEW**D**KNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	471.
HS9_DS B7	HGEGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCGGGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVT ITCQASQDVKTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQRYSLWRT FGQGTKLEIK	472.
GLP-1 analogue	HGEGTFTSDVSSYLEEQAAKEFIAWLVKGG	473.
NGS#1	HGEGTFTSDVSSYLEEQ**N**A**S**EFIAWLVKGG	474.
NGS#2	HGEGTFTSDVSSYLEEQAAKEFIAWLV**N**G**S**	475.
NGS#3	HGEGTFTSDVSSYLEEQAAKEFIAWLVK**N**G	476.
NGS#4	HGEGTFTSDVSSYLEEQAAKEFIAWLVKG**N**	477.

NGS#5	HGEGTFTSDVSSYLEEQAAKEFIAWLVKGG	478.
NGS#6	HGEGTFTSDVSSYLEEQAAKEFIAWLVKGG	479.
NGS#7	HGEGTFTSDVSSYLEEQAAKEFIA**N**L**S**KGG	480.
NGS#8	HGEGTFTSDVSSYLEEQAAKEFIA**N**L**T**KGG	481.
Linker	GGGGGSGGGGSGGGGSA	482.
Linker	**S**GGGGSGGGGSGGGGSA	483.
Linker	G**S**GGGSGGGGSGGGGSA	484.
Linker	**N**G**S**GGSGGGGSGGGGSA	485.
Linker	G**N**G**S**GSGGGGSGGGGSA	486.
PC9_2_DSB#1 VL (Version B) (with K at end of variable light chain)	***HGEGTFTSCLSKQMEEEAVRLFIEWLKNGGPSSGAPPPSGGGGGGGGGGGGCGG***GGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVTITC**KASQDVHTAVA**WYQQKPGKAPKLLIY**HASYRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQRYSLWRT**FGQGTKLEIK	487.
PC9_2_DSB#3 VL (Version B) (with K at end of variable light chain)	***HGEGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPGC***GGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVTITC**KASQDVHTAVA**WYQQKPGKAPKLLIY**HASYRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQRYSLWRT**FGQGTKLEIK	488.
PC9_2_NGS#7 VL (Version B) (with	***HGEGTFTSDVSSYLEEQAAKEFIANLSKGGG***GGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVTITC**KASQDVHTAVA**WYQQKPGKAPKLLIY**HASYRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQRYSLWRT**FGQGTKLEIK	489.

K at end of variable light chain)		
PC9_2_DSB#7 VL (Version B) (with K at end of variable light chain)	***HGEGTFTSDLSKQMEEECVRLFIEWLKNGGPSSGAPPPGCG***GGGGSGGGGSGGGGSADIQMTQSPSSLSASVGDRVTITC**KASQDVHTAVA**WYQQKPGKAPKLLIY**HASYRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQRYSLWRT**FGQGTKLEIK	490.
Engineered PCSK9 antibody VH	EVQLVESGGGLVKPGGSLRLSCAASGFPFSKLGMVWVRQAPGKGLEWVSTISSGGGYTYYPDSVKGRFTISRDNAKNSLY LQMNSLRAEDTAVYYCAREGISFQGGTYTYVMDYWGQGTLVTVSS	491.
Engineered PCSK9 antibody VL	DIVMTQSPLSLPVTPGEPASISCRSSKSLLHRNGITYSYWYLQKPGQSPQLLIYQLSNLASGVPDRFSGSGSGTDFTLKI SRVEAEDVGVYYCYQNLELPLTFGQGTKVEIK	492.
Engineered PCSK9 antibody VH CDR1	GFPFSKLGMV	493.
Engineered PCSK9 antibody	TISSGGGYTYYPDSVK	494.

VH CDR2		
Engineered PCSK9 antibody VH CDR3	EGISFQGGTYTYVMDY	495.
Engineered PCSK9 antibody VL CDR1	RSSKSLLHRNGITYSY	496.
Engineered PCSK9 antibody VL CDR2	QLSNLAS	497.
Engineered PCSK9 antibody VL CDR3	YQNLELPLT	498.

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DESCRIPTION OF THE EMBODIMENTS

I. Anti-PCSK9~GLP-1 Fusion Molecules

[0101] The present disclosure is directed to fusions of antibodies (e.g., anti-PCSK9 antibodies or antigen-binding fragments thereof) with a GLP-1 moiety.

[0102] In one embodiment the fusion is constructed as:

GLP-1 moiety—Linker—Antibody Light Chain.

or

GLP-1 moiety-Linker-Antibody Heavy Chain

[0103] The fusion protein can be constructed as a genetic fusion. Alternatively, the fusion protein may be constructed as a chemical conjugate, such as through a cysteine:cysteine disulfide bond.

[0104] Other arrangements of the GLP-1 moiety and antibody portion are also within the scope herein.

A. Antibodies and Antigen-Binding Fragments Thereof

1. Anti-PCSK9 Portion

[0105] The antibody portion may be an anti-PCSK9 antibody or an antigen-binding fragment thereof. In one embodiment, the anti-PCSK9 portion provides an LDLc (bad cholesterol) lowering effect.

[0106] In one embodiment, the anti-PCSK9 VL portion may be SEQ ID NO: 2 (PC9_2_HS9). In another embodiment, the anti-PCSK9 VL portion may be an antigen binding portion of SEQ ID NO: 2. In one embodiment, the anti-PCSK9 VL portion may comprise all six CDRs of SEQ ID NO: 2. In one embodiment, the anti-PCSK9 VH portion may be a pH dependent version of the antibody, as shown in SEQ ID NO: 5.

[0107] In one embodiment, the antibody or antigen-binding fragment thereof may be pH dependent, such that the antibody binding to the antigen is pH dependent. This can be used to modify half-life of the antibody and/or antigen. By modifying the antibody half-life, in one embodiment, we mean lengthening the half-life. By modifying the antibody half-life, in one embodiment, we mean shortening the half-life. In one embodiment, the antibody half-life may be modified (lengthened or shortened) to maximize the stability of it fusion partner (i.e., GLP-1). By modifying the antigen half-life we may also mean that the antigen-antibody complex can change the antigen's half-life, such as through antibody-mediated degradation (shortening the half-life) or by protecting the antigen from the typical degradation process (lengthening the half-life). In one instance, antibodies may have a higher affinity for the antigen at pH 7.4 as compared to endosomal pH (i.e., pH 5.5-6.0), such that the K_D ratio at pH 5/5/pH 7.4 or at pH 6.0/pH 7.2 is 2 or more. Methods of engineering pH dependent antibodies are described in US 2011/0229489 and 2014/0044730, which are incorporated by reference herein.

[0108] In one embodiment the anti-PCSK9 portion provides sustained suppression of free PCSK9. In one embodiment, is provides at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% suppression.

[0109] In one embodiment, an anti-PCSK9 antibody or antigen binding fragment thereof capable of specifically binding PCSK9 comprises:

a. a heavy chain variable region comprising a sequence which is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 1, 5, 8, or 10; and

b. a light chain variable region comprising a sequence which is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 2, 6, 9 or 11.

[0110] In one embodiment, an anti-PCSK9 antibody or antigen binding fragment thereof capable of specifically binding PCSK9 comprises:

a. a heavy chain variable region CDR1 sequence comprising a sequence which has one mutation compared to SEQ ID NO: 14;

b. a heavy chain variable region CDR2 sequence comprising a sequence which has one or two mutations compared to SEQ ID NO: 15, 16, 17, or 18;

c. a heavy chain variable region CDR3 sequence comprising a sequence which is one mutation compared to SEQ ID NO: 19.

d. a light chain variable region CDR1 sequence comprising a sequence which has one or two mutations compared to SEQ ID NO: 20, 21, 22, or 23;

e. a light chain variable region CDR2 sequence comprising a sequence which has one mutation compared to SEQ ID NO: 24 or 25; and

f. a light chain variable region CDR3 sequence comprising a sequence which has one mutation compared to SEQ ID NO: 26.

[0111] In one embodiment, an anti-PCSK9 antibody or antigen binding fragment thereof capable of specifically binding PCSK9 comprises:

a. a heavy chain variable region CDR1 sequence comprising SEQ ID NO: 14;

b. a heavy chain variable region CDR2 sequence comprising SEQ ID NO: 15, 16, 17, or 18;

c. a heavy chain variable region CDR3 sequence comprising SEQ ID NO: 19.

d. a light chain variable region CDR1 sequence comprising SEQ ID NO: 20, 21, 22, or 23;

e. a light chain variable region CDR2 sequence comprising SEQ ID NO: 24 or 25; and

f. a light chain variable region CDR3 sequence comprising SEQ ID NO: 26.

[0112] In another embodiment, the anti-PCSK9 portion may comprise an anti-PCSK9 antibody or antigen-binding fragment as described in any of US 8,030,457, US 8,062,640, US

8,357,371, US 8,168,762, US 8,563,698, US 8,829,165, US 8,859,741, US 8,188,233, WO 2012/088313, US 2012/0195910, US 8,530,414, US 2013/0189278, US 8,344,144, US 2011/0033465, US 8,188,234, US 8,080,243, US 2011/0229489, US 2010/0233177, US 2013/315927 and US 2013/0071405. Each of these references is incorporated by reference for the sequence and description of anti-PSCK9 antibodies and antigen-binding fragments thereof.

[0113] In one embodiment, the anti-PCSK9 portion may comprise a heavy and light chain variable region chosen from the sequences in Group B of Table 1 above. Alternatively, the anti-PCSK9 portion may comprise a heavy and light chain variable region with CDRs identical to a heavy and light chain variable region from Group B of Table 1 above. Additionally, the anti-PCSK9 portion may comprise a heavy chain variable region chosen from SEQ ID NOS: 53, 55, 57, 59, 61, or 63 and a light chain variable region chosen from SEQ ID NOS: 54, 56, 58, 60, 62, or 64. Alternatively, the anti-PCSK9 portion may comprise heavy chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 53, 55, 57, 59, 61, or 63 and light chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 54, 56, 58, 60, 62, or 64.

[0114] In one embodiment, the anti-PCSK9 portion may comprise a heavy and light chain variable region chosen from the sequences in Group C of Table 1 above. Alternatively, the anti-PCSK9 portion may comprise a heavy and light chain variable region with CDRs identical to a heavy and light chain variable region from Group C of Table 1 above. Additionally, the anti-PCSK9 portion may comprise a heavy chain variable region chosen from SEQ ID NOS: 65-95 and a light chain variable region chosen from SEQ ID NOS: 96-126. Alternatively, the anti-PCSK9 portion may comprise heavy chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 65-95 and light chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 96-126.

[0115] In one embodiment, the anti-PCSK9 portion may comprise a heavy and light chain variable region chosen from the sequences in Group D of Table 1 above. Alternatively, the anti-

PCSK9 portion may comprise a heavy and light chain variable region with CDRs identical to a heavy and light chain variable region from Group D of Table 1 above. Additionally, the anti-PCSK9 portion may comprise a heavy chain variable region chosen from SEQ ID NOS: 127-218 and a light chain variable region chosen from SEQ ID NOS: 219-311. Alternatively, the anti-PCSK9 portion may comprise heavy chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 127-218 and light chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 219-311.

[0116] In one embodiment, the anti-PCSK9 portion may comprise a heavy and light chain variable region chosen from the sequences in Group E of Table 1 above. Alternatively, the anti-PCSK9 portion may comprise a heavy and light chain variable region with CDRs identical to a heavy and light chain variable region from Group E of Table 1 above. Additionally, the anti-PCSK9 portion may comprise a heavy chain variable region chosen from SEQ ID NOS: 312-317 and a light chain variable region chosen from SEQ ID NOS: 318-323. Alternatively, the anti-PCSK9 portion may comprise heavy chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 312-317 and light chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 318-323.

[0117] In one embodiment, the anti-PCSK9 portion may comprise a heavy and light chain variable region chosen from the sequences in Group F of Table 1 above. Alternatively, the anti-PCSK9 portion may comprise a heavy and light chain variable region with CDRs identical to a heavy and light chain variable region from Group F of Table 1 above. Additionally, the anti-PCSK9 portion may comprise a heavy chain variable region chosen from SEQ ID NO: 324 and a light chain variable region chosen from SEQ ID NO: 325. Alternatively, the anti-PCSK9 portion may comprise heavy chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 324 and light chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 325.

[0118] In one embodiment, the anti-PCSK9 portion may comprise a heavy and light chain variable region chosen from the sequences in Group G of Table 1 above. Alternatively, the anti-

PCSK9 portion may comprise a heavy and light chain variable region with CDRs identical to a heavy and light chain variable region from Group G of Table 1 above. Additionally, the anti-PCSK9 portion may comprise a heavy chain variable region chosen from SEQ ID NOS: 326, 339, 340, or 343-400 and a light chain variable region chosen from SEQ ID NOS: 327, 341, or 342. Alternatively, the anti-PCSK9 portion may comprise heavy chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 326, 339, 340, or 343-400 and light chain CDR1, CDR2, and CDR3 from any one of SEQ ID NOS: 327, 341, or 342. Further, the anti-PCSK9 portion may comprise heavy chain CDR1, CDR2, and CDR3 from SEQ ID NOS: 328 (HC CDR1), 329 (HC CDR2), 331 (HC CDR2), 330 (HC CDR3), 332 (HC CDR3) and light chain CDR1, CDR2, and CDR3 from SEQ ID NOS: 333 (LC CDR1), 334 (LC CDR2), 335 (LC CDR3), 336 (LC CDR1), 337 (LC CDR2), and 338 (LC CDR3).In one embodiment, the anti-PCSK9 portion may comprise a heavy variable region comprising the amino acid sequence of SEQ ID NO: 491 and light chain variable region comprising the amino acid sequence of SEQ ID NO: 492. Alternatively, the anti-PCSK9 portion may comprise heavy chain CDR1, CDR2, and CDR3 from SEQ ID NOS: 493-495 and light chain CDR1, CDR2, and CDR3 from SEQ ID NOS: 496-498.

[0119] In other embodiments, the antibody portion may comprise antibodies other than an anti-PCSK9 antibody (e.g., an anti-B7-H1 antibody). In one embodiment, the anti-B7-H1 antibody may comprise a heavy variable region comprising the amino acid sequence of SEQ ID NO: 422 and light chain variable region comprising the amino acid sequence of SEQ ID NO: 423.

2. Antibody or Antigen-Binding Fragments

[0120] As used herein, the term antibody or antigen-binding fragment thereof is used in the broadest sense. It may be man-made such as monoclonal antibodies (mAbs) produced by conventional hybridoma technology, recombinant technology and/or a functional fragment thereof.

It may include both intact immunoglobulin molecules for example a polyclonal antibody, a monoclonal antibody (mAb), a monospecific antibody, a bispecific antibody, a polyspecific antibody, a human antibody, a humanized antibody, an animal antibody (e.g. camelid antibody), chimeric antibodies, as well as portions, fragments, regions, peptides and derivatives thereof (provided by any known technique, such as, but not limited to, enzymatic cleavage, peptide synthesis, or recombinant techniques), such as, for example, immunoglobulin devoid of light chains, Fab, Fab', F $(ab')_2$, Fv, scFv, antibody fragment, diabody, Fd, CDR regions, or any portion or peptide sequence of the antibody that is capable of binding antigen or epitope. In one embodiment, the functional part is a single chain antibody, a single chain variable fragment (scFv), a Fab fragment, or a $F(ab')_2$ fragment.

[0121] An antibody or functional part is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. Antibody fragments or portions may lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. Examples of antibody may be produced from intact antibodies using methods well known in the art, for example by proteolytic cleavage with enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). Portions of antibodies may be made by any of the above methods, or may be made by expressing a portion of the recombinant molecule. For example, the CDR region(s) of a recombinant antibody may be isolated and subcloned into an appropriate expression vector.

[0122] In one embodiment, an antibody or functional part is a human antibody. The use of human antibodies for human therapy may diminish the chance of side effects due to an immunological reaction in a human individual against nonhuman sequences. In another embodiment, the antibody or functional part is humanized. In another embodiment, an antibody or

functional part is a chimeric antibody. This way, sequences of interest, such as for instance a binding site of interest, can be included into an antibody or functional part.

[0123] In one embodiment, the antibody may have an IgG, IgA, IgM, or IgE isotype. In one embodiment, the antibody is an IgG. In one aspect, the anti-PCSK9 antibody or antigen-binding fragment thereof may be an IgG1.

3. Modifications to the Constant Domain

[0124] In one embodiment, the anti-PCSK9 antibody or antigen binding fragment comprises an Fc region. It will be understood that Fc region as used herein includes the polypeptides comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM Fc may include the J chain. For IgG, Fc comprises immunoglobulin domains Cgamma2 and Cgamma3 (Cγ2 and Cγ3) and the hinge between Cgamma1 (Cγ1) and Cgamma2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat et al. (1991, NIH Publication 91-3242, National Technical Information Service, Springfield, VA). The "EU index as set forth in Kabat" refers to the residue numbering of the human IgG1 EU antibody as described in Kabat et al. *supra*. Fc may refer to this region in isolation, or this region in the context of an antibody, antibody fragment, or Fc fusion protein.

[0125] In one embodiment, the anti-PCSK9 antibody or antigen-binding portion thereof has a variant Fc region having reduced effector function (e.g., reduced ADCC and/or CDC). In one embodiment, the Fc region has no detectable effector function. In one embodiment, the Fc region comprises at least one non-native amino acid at one or more positions chosen from 234, 235, and

331, as numbered by the EU index as set forth in Kabat. In a specific embodiment, the present invention provides an Fc variant, wherein the Fc region comprises at least one non-native amino acid chosen from 234F, 235F, 235Y, and 331S, as numbered by the EU index as set forth in Kabat. In a further specific embodiment, an Fc variant of the invention comprises the 234F, 235F, and 331S amino acid residues, as numbered by the EU index as set forth in Kabat. In another specific embodiment, an Fc variant of the invention comprises the 234F, 235Y, and 331S amino acid residues, as numbered by the EU index as set forth in Kabat. In a particular embodiment, the anti-PCSK9 antibody or antigen-binding portion thereof has a variant Fc region, wherein the variant comprises a phenylalanine (F) residue at position 234, a phenylalanine (F) residue or a glutamic acid (E) residue at position 235 and a serine (S) residue at position 331, as numbered by the EU index as set forth in Kabat. Such mutation combinations are hereinafter referred to as the triple mutant (TM).

[0126] The serine228proline mutation (S228P), as numbered by the EU index as set forth in Kabat, hereinafter referred to as the P mutation, has been reported to increase the stability of a particular IgG4 molecule (Lu et al., J Pharmaceutical Sciences 97(2):960-969, 2008). Note: In Lu et al. it is referred to as position 241 because therein they use the Kabat numbering system, not the "EU index" as set forth in Kabat.

[0127] This P mutation may be combined with L235E to further knock out ADCC. This combination of mutations is hereinafter referred to as the double mutation (DM).

B. GLP-1 Moiety

[0128] The fusion molecule contains a GLP-1 moiety. GLP-1 may also be referenced by the synonym glucagon-like peptide-1. By GLP-1 we also reference Exendin-4, which is a GLP-1 analog. In one embodiment, the GLP-1 moiety provides glucose control and/or weight loss benefits.

[0129] In one embodiment, the full length GLP-1 molecule may be used in the fusion protein. In another embodiment, a fragment of GLP-1 may be used as a GLP-1 moiety in the fusion protein.

[0130] In one embodiment, the GLP-1 moiety has a pair of cysteine residues that allows for a disulphide bridge. In one embodiment, the cysteine is an engineered cysteine compared to the parental sequence. In one embodiment, the cysteine is an E18C mutation.

[0131] In one embodiment, the GLP-1 potency is reduced at the human GLP-1 receptor compared to wild type GLP-1 (e.g., SEQ ID NO: 29) or a GLP-1 analog (e.g., SEQ ID NO: 12). In another embodiment, the GLP-1 potency at the human GLP-1 receptor is at least about 10X, 20X, 30X, 40X, 50X, 60X, 100X, 125X, 150X, 175X, 200X, or 225X lower than wildtype GLP-1 or a GLP-1 analog. In another embodiment, the potency is reduced by similar amounts as compared to dulaglutide. The GLP-1 potency may be reduced to allow for saturation of PCSK9 while reducing side effects. In one embodiment, the GLP-1 moiety may have at least one mutation that reduces the potency of the GLP-1 moiety. In one embodiment, this offers benefits of reducing side effects, including, but not limited to nausea. In one embodiment, the mutation is a point mutation. In one embodiment, the point mutation is chosen from V19A, G2V, E15A, or L26I with respect to Exendin-4.

[0132] In one embodiment, the GLP-1 moiety is comprises any one of SEQ ID NOS: 3, 7, 12, 13, or 28-42. In another embodiment, the GLP-1 moiety is a fragment of any one of SEQ ID NOS: 3, 7, 12, 13, or 28-42 comprising at least 10, 15, 20, or 25 amino acids. In a further embodiment, the GLP-1 moiety comprises a sequence which is at least at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to any one of SEQ ID NOS: 3, 7, 12, 13, or 28-42. In a further embodiment, the GLP-1 moiety comprises a sequence which has 1, 2, 3, 4, 5, or 6 mutations as compared to any one of SEQ ID NOS: 3, 7, 12, 13, or 28-42.

C. Fusion and Linkers

[0133] In one embodiment, the GLP-1 moiety is fused directly or indirectly to the light chain of the anti-PCSK9 antibody or antigen binding fragment. In another embodiment, the GLP-1 moiety is fused directly or indirectly to the heavy chain of the anti-PCSK9 antibody or antigen binding fragment.

[0134] In one embodiment, a linker may be used to construct a fusion between the anti-PCSK9 antibody or antigen-binding fragment and the GLP-1 moiety. In another embodiment, the anti-PCSK9 antibody or antigen-binding fragment may be directly conjugated to the GLP-1 moiety.

[0135] If a linker is used, the linker may be chosen from any suitable linker for fusion proteins. In one embodiment, the linker may comprise a GGGGS (SEQ ID NO: 27) repeat, either alone or in combination with other amino acids, either as one, two, three, or four sets of repeats. In one embodiment, the linker may comprise other combinations of G and S, either alone or in combination with other amino acids. In some embodiments, the linker has a C-terminal Alanine (A).

[0136] In one embodiment, a specific linker may be chosen from GGGGSGGGGSGGGGSA (SEQ ID NO: 4). In one embodiment, the linker allows for a disulfide bridge to form between the C terminus of the GLP-1 moiety and another portion of the GLP-1 molecule, but not with the antibody portion. In such an instance, a linker with limited flexibility may prevent undesired disulfide bridging to the antibody portion.

[0137] In one embodiment, the linker is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 4.

[0138] In one embodiment, the cysteine:cysteine disulfide bridge is formed by making a cysteine substitution mutation in the GLP-1 moiety. In one embodiment, the mutation is E18C.

II. Nucleic Acids Encoding Fusion Molecules

[0139] The present embodiments further provides an isolated, synthetic, or recombinant nucleic acid sequence encoding any of the fusion molecules described in section I above. Such nucleic acids encode the heavy and light chain sequences set forth herein. Alternatively, such nucleic acids include the anti-PCSK9 antibody or antigen-binding portion fused to the GLP-1 moiety portion. Due to the degeneracy of the nucleic acid code, multiple nucleic acids will encode the same amino acid and all are encompassed herein.

III. Methods of Making Fusion Molecules, Formulation, and Pharmaceutical Compositions

[0140] One embodiment includes a method of producing the fusion molecule by culturing host cells under conditions wherein a nucleic acid is expressed to produce the fusion molecule, followed by recovering the fusion molecule. A variety of cell lines may be used for expressing the fusion molecule, including, but not limited to, mammalian cell lines. In one embodiment, the cell lines may be human. In another embodiment, bacterial or insect cell lines may be used. In one embodiment, the cell lines include Chinese hamster ovary (CHO) cells, variants of CHO cells (for example DG44), 293 cells, and NS0 cells. In another embodiment, cell lines include VERY, BHK, Hela, COS, MDCK, 293F, 293T, 3T3, W138, BT483, Hs578T, Sp2/0, HTB2, BT2O, T47D, CRL7O3O, and HsS78Bst cells.

[0141] Recombinant expression utilizes construction of an expression vector containing a polynucleotide that encodes the fusion molecule. Once a polynucleotide has been obtained, a vector for the production of the fusion molecule may be produced by recombinant DNA technology well known in the art. Expression vectors may include appropriate transcriptional and translational control signals. This may be accomplished using *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. In one embodiment, a replicable vector comprises a

nucleic acid sequence encoding an antibody or functional part operably linked to a heterologous promoter.

[0142] A variety of host-expression vector systems may be utilized to express the fusion molecule as described in U.S. Pat. No. 5,807,715. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus, are an effective expression system for antibodies (Foecking et al., Gene, 45:101 (1986); and Cockett et al., Bio/Technology, 8:2 (1990)). In addition, a host cell strain may be chosen which modulates the expression of inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the protein of the invention. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used.

[0143] In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the fusion molecule being expressed. For example, when a large quantity of such fusion molecule is to be produced, for the generation of pharmaceutical compositions comprising the fusion molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., EMBO, 12:1791 (1983)), in which the coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109

(1985); Van Heeke & Schuster, 1989, J. Biol. Chem., 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione-S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to glutathione-agarose affinity matrix followed by elution in the presence of free glutathione. The pGEX vectors are designed to introduce a thrombin and/or factor Xa protease cleavage sites into the expressed polypeptide so that the cloned target gene product can be released from the GST moiety.

[0144] In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The protein coding sequence may be cloned individually into non-essential regions (for example, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example, the polyhedrin promoter).

[0145] In mammalian host cells, a number of virus based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion into a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody or functional part in infected hosts (e.g., see, Logan & Shenk, Proc. Natl. Acad. Sci. USA, 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody or functional part coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon should generally be in frame with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both

natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, e.g., Bittner et al., Methods in Enzymol., 153:51-544(1987)).

[0146] Stable expression can be used for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express the fusion molecule may be generated. Host cells can be transformed with an appropriately engineered vector comprising expression control elements (e.g., promoter, enhancer, transcription terminators, polyadenylation sites, etc.), and a selectable marker gene. Following the introduction of the foreign DNA, cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells that stably integrated the plasmid into their chromosomes to grow and form foci which in turn can be cloned and expanded into cell lines. Plasmids that encode the fusion molecule can be used to introduce the gene/cDNA into any cell line suitable for production in culture.

[0147] A number of selection systems may be used, including, but not limited to, the herpes simplex virus thymidine kinase (Wigler et al., Cell, 11:223 (1977)), hypoxanthineguanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA, 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell, 22:8-17 (1980)) genes can be employed in tk-, hgprt- or aprT-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA, 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA, 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA, 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIB TECH

11(5):155-2 15 (1993)); and hygro, which confers resistance to hygromycin (Santerre et al., Gene, 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds.), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., 1981, J. Mol. Biol., 150:1.

[0148] Once the fusion molecule has been produced by recombinant expression, it may be purified by any method known in the art for purification, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigens Protein A or Protein G, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

[0149] Further provided are compositions, *e.g.*, pharmaceutical compositions, that contain an effective amount of a dual active fusion molecule as provided herein, formulated for the treatment of metabolic diseases, *e.g.*, Type 2 diabetes.

[0150] Compositions of the disclosure can be formulated according to known methods. Suitable preparation methods are described, for example, in *Remington's Pharmaceutical Sciences*, 19th Edition, A.R. Gennaro, ed., Mack Publishing Co., Easton, PA (1995), which is incorporated herein by reference in its entirety. Composition can be in a variety of forms, including, but not limited to an aqueous solution, an emulsion, a gel, a suspension, lyophilized form, or any other form known in the art. In addition, the composition can contain pharmaceutically acceptable additives including, for example, diluents, binders, stabilizers, and preservatives. Once formulated, compositions of the disclosure can be administered directly to the subject.

[0151] Carriers that can be used with compositions of the disclosure are well known in the art, and include, without limitation, *e.g.*, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, and polyamino acids such as poly L-lysine, poly L-glutamic acid, influenza, hepatitis B virus core protein, and the like. A variety of aqueous carriers can be used, *e.g.*, water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. Compositions can be sterilized by conventional, well known sterilization techniques, or can be sterile filtered. A resulting composition can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. Compositions can contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamineoleate, etc.

IV. Methods for Use of the Anti-PCSK9~GLP-1 Fusion Molecule and Kits

[0152] The anti-PCSK9~GLP-1 fusion may be used to treat diabetes or TType 2 diabetes. In one embodiment, the patient has TType 2 diabetes. In one embodiment, the patient has a high cardiovascular risk profile. In another embodiment, the patient has both TType 2 diabetes and a high cardiovascular risk profile. By high cardiovascular risk profile, it means that the patient is at higher risk for a cardiovascular event due to one or more factors: high cholesterol, high LDL cholesterol, low HDL cholesterol, high blood pressure, atherosclerosis, obesity, prior cardiovascular event (including angina, heart attack, transient ischemic attack, stroke, etc.), family history of cardiovascular event, smoking, high triglycerides, lack of physical activity, poorly controlled blood sugars, and the like.

[0153] In other embodiments, the anti-PCSK9~GLP-1 fusion may be used to treat other disease including but not limited to NASH, obesity, hypercholesterolemia, and major adverse

cardiovascular events (MACE) including but not limited to acute coronary syndrome (ACS), stroke, heart failure, and malignant dysrhythmia.

[0154] In one embodiment, the fusion molecule has increased stability upon administration. In one embodiment, the increased stability is demonstrated by comparing it to a benchmark control compound dulaglutide, a GLP-1 analog fused with an Fc fragment, in an in vivo administration to mice.

[0155] In one embodiment, the fusion molecule as increased potency at the GLP-1 receptor. In one embodiment, the decreased potency is demonstrated at the human GLP-1 receptor over the benchmark control compound dulaglutide and/or wild type GLP-1.

[0156] In some embodiments, the fusion molecule promotes weight loss in a subject.

[0157] In one embodiment, the fusion molecule is administered by injection.

[0158] In other embodiments, the present disclosure provides kits comprising dual active fusion molecules, which can be used to perform the methods described herein. In certain aspects, a kit comprises a dual active fusion molecule disclosed herein in one or more containers. A kit as provided herein can contain additional compositions for combination therapies. One skilled in the art will readily recognize that the disclosed dual active fusion molecules can be readily incorporated into one of the established kit formats that are well known in the art.

[0159] Reference will now be made in detail to the present exemplary embodiments, examples of which are illustrated in the accompanying drawings. Wherever possible, the same reference numbers will be used throughout the drawings to refer to the same or like parts. Other embodiments will be apparent to those skilled in the art from consideration of the specification and practice disclosed herein. The embodiments are further explained in the following examples. These examples do not limit the scope of the claims, but merely serve to clarify certain embodiments. It is

intended that the specification and examples be considered as exemplary only, with a true scope and spirit being indicated by the following claims.

EXAMPLES

Example 1. Antibody Optimisation

[0160] The anti-PCSK9 antibody PC9#2 (SEQ ID NOs. 8 and 9 for the variable heavy and light chains respectively) has been optimised to:

[0161] 1_ Reduce the immunogenicity risk by reverting amino acids to the ones corresponding to the closest human germline sequence without significantly impacting the binding to PCSK9 antigen.

[0162] 2_ Remove pH-dependent binding to PCSK9 by mutating histidine residues VH_52, VL_30 and VL_50 (Kabat numbering).

[0163] 3_ Improve affinity for human PCSK9 at physiological pH in order to efficiently engage with the target and achieve sufficient free PCSK9 suppression following administration of PCSK9/GLP-1 peptide antibody fusion molecules.

A) Germlining

[0164] The amino acid sequences of the VH and VL domains of the anti-PCSK9 antibody PC9#2 were aligned to the known human germline sequences in the VBASE database (Tomlinson, 1997; http://vbase.mrc-cpe.cam.ac.uk/), and the closest human germline was identified by sequence similarity to be 1-46 (DP-7) (SEQ ID 417) and VK1 O18 O8 (DPK1) (SEQ ID 418) for the variable heavy and light chains respectively. Figures 1E and 1F are showing an alignment of PC9#2 variable domains with those germline sequences.

[0165] A structure model of the anti-PCSK9 PC9#2 antibody in complex with human PCSK9 antigen has been generated using the primary amino acid sequence of PC9#2 variable domains and previously described crystal structure of human PCSK9 in complex with another anti-

PCSK9 antibody deposited in the Protein Data Bank using PBD ID code 3SQO (Liang et al., 2012, J. Pharm. Exp. Ther., Vol. 340, p228-236).

[0166] Using that structure model, the following residues were identified as non-contacting with PCSK9 antigen but nonetheless solvent-exposed: Arg56, Asn58, Glu61, Lys64 and Ser65 in the variable heavy chain as well as Arg24 in the variable light chain (Kabat numbering). Because those residues are different from the closest germline sequences and might be solvent-exposed, they can present some immunogenicity risks. Without being bound by theory, mutating those residues should not significantly impact the ability of the antibody to strongly bind PCSK9 as they should not contribute to the interaction network between the antibody and its antigen.

[0167] Mutations R56S, N58S, E61Q, K64Q and S65G were then introduced in PC9#2 heavy chain sequence as well as K24Q in the light chain using standard molecular biology techniques to generate the antibody PC9#2_FG of SEQ ID 5 and 6 for the variable heavy and light chains respectively.

[0168] Anti-PCSK9 PC9#2 and PC9#2_FG were produced as human IgG1-TM antibodies as described in Example 2 and characterised for binding to human PCSK9 using Biacore as described in Example 17. Kinetic parameters at pH 7.4 for those compounds are summarised in Table 2. Both antibodies exhibit similar on-rate, off-rate and affinity for human PCSK9 demonstrating that the germlining mutagenesis had no impact on antigen binding.

Table 2: Kinetic parameters for human PCSK9 at physiological pH of germlined and non-germlined version of PC9#2antibody

Compound	ka (1/Ms)	kd (1/s)	KD (M)
PC9#2	2.70E+05	5.10E-04	1.89E-09
PC9#2_FG	3.03E+05	5.40E-04	1.78E-09

B) Removing pH dependent binding and improving affinity for human PCSK9

[0169] Anti-PCSK9 antibodies PC9#2 and PC9#2_FG exhibit pH-dependent binding properties as they bind strongly to human PCSK9 antigen at physiological pH but rapidly dissociate from their target at acidic pH. That feature should enable the antibodies to dissociate from PCSK9 in the acidic compartment of endosome in order to be recycled at cell surface rather than being sent to lysosome for degradation. This should ultimately translate into a longer antibody *in vivo* half-life.

[0170] Table 3 are comparing off-rate (kd) for those two compound with human PCSK9 at pH7.4 (physiological) and 6.0 (acidic) determined by Biacore as described in Example 17 but with the following modifications. After antibody capture onto the CM5 chip, human PCSK9 antigen diluted to concentrations ranging from 1 nM to 200 nM in running buffer pH7.4 (10 mM sodium phosphate pH 7.4, 150 mM sodium chloride, 1 mg/mL BSA, 0.05% Tween20) were injected for 10 minutes. After the association phase, running buffer at pH7.4 or pH6.0 were injected for 10 minutes dissociation phase. Global dissociation rates were calculated using a 1:1 binding kinetics model.

Table 3: Dissociation constant (kd) at physiological and acidic pH of germlined and non-germlined version of PC9#2antibody.

Compound	kd (1/s) pH7.4	kd (1/s) pH6.0	kd ratio
PC9#2	5.10E-04	3.24E-03	6.4
PC9#2_FG	5.40E-04	6.47E-03	12

[0171] Long antibody *in vivo* half-life is not desirable for PCSK9/GLP-1 fusion molecules as it might lead to the accumulation of drug metabolites able to bind PCSK9 but degraded in the GLP-1 analogue peptide and thus unable to activate the GLP-1 receptor.

[0172] In addition, genetic fusion of a GLP-1 analogue peptide in front of the light chain of the anti-PCSK9 PC9#2 was slightly impacting the affinity of the compound for human PCSK9 (19nM) compared to PC9#2 antibody (7nM) as shown in Example 5, Table 11. Affinity maturation

of PC9#2_FG was then required in order to counterbalance the negative impact of GLP-1 analogue light chain fusion on affinity for human PCSK9 antigen.

[0173] To remove pH-dependent binding, histidine residues in position 52 of the heavy chain as well as in position 30 and 50 of the light chain need to be mutated. Based on the structure model described above and subsequent analysis of PC9#2 in complex with human PCSK9, the following mutations were identified as potentially beneficial for antibody binding to PCSK9 and could lead to an affinity improvement: heavy chain H52K or H52S, light chain H30Y, H30K or H30S and light chain H50Y or H50S.

[0174] Combination of all those mutations in the sequence of PC9#2_FG were generated using standard molecular biology techniques in order to produce optimised antibodies. Table 4 is summarising the different compounds resulting from the combination experiment.

Table 4. PC9#2_FG mutations to generate optimised antibodies

#	VH_H52	VL_H30	VL_H50	Antibody name	SEQ ID
1	K	Y	Y	HS1	424 and 425
2	K	Y	S	HS2	426 and 427
3	K	K	Y	HS3	428 and 429
4	K	K	S	HS4	430 and 431
5	K	S	Y	/	
6	K	S	S	HS5	432 and 433
7	S	Y	Y	HS6	434 and 435
8	S	Y	S	HS7	436 and 437
9	S	K	Y	HS8	438 and 439
10	S	K	S	HS9	1 and 2
11	S	S	Y	/	
12	S	S	S	HS10	442 and 443

[0175] Combination of H30S and H50Y mutations in PC9#2_FG light chain failed to deliver and compound #5 and #11 have subsequently not been generated.

[0176] Antibodies were produced as human IgG1-TM as described in Example 2 and tested for their ability to block the binding of PC9#2 to human PCSK9 using an epitope competition assay as described in Example 3. Data are summarised in Table 5 and show that HS7, HS9 and HS10 have an IC50 at least 10-fold lower compared to the parent antibody PC9#2_FG suggesting that those antibodies may have a significantly better affinity for PCSK9 than PC9#2_FG.

Table 5. Inhibition of PC9#2 binding to human PCSK9 using anti-PCSK9 antibodies as competition reagent.

Antibody	IC50 (M)	Ratio over PC9#2_FG
HS1	5.6E-10	1.3
HS2	9.3E-11	7.8
HS3	7.0E-10	1.0
HS4	1.2E-10	6.1
HS5	2.2E-10	3.3
HS6	1.7E-10	4.3
HS7	6.3E-11	11.6
HS8	1.6E-10	4.6
HS9	6.6E-11	11.1
HS10	7.0E-11	10.4
PC9#2_FG	7.3E-10	1.0

[0177] Antibody HS9 has been further characterised and compared to the parental antibody PC9#2_FG for its binding parameters to human PCSK9 at physiological pH by Biacore as described in Example 17. As shown in Table 6, engineered anti-PCSK9 antibody HS9 displays a 3-fold improvement in affinity for human PCSK9 compared to PC9#2_FG at physiological pH, mainly due to a reduction in off-rate (kd).

Table 6. Kinetic parameters for human PCSK9 at physiological pH of engineered antibody HS9 compared to PC9#2_FG

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
PC9#2_FG	3.09E+05	6.57E-04	2.13E-09
HS9	3.81E+05	2.57E-04	6.75E-10

[0178] In a separate experiment, pH dependent binding of HS9 anti-PCSK9 antibody has been compared to PC9#2_FG using Biacore as described above. Table 7 is showing the dissociation constant (kd) at physiological and acidic pH for those two compounds. Contrary to PC9#2_FG which dissociates more rapidly at pH6.0 than at pH7.4, HS9 displays a lower kd at acidic than at

physiological pH demonstrating that it is dissociating from PCSK9 more slowly at pH6.0 than at pH7.4.

Table 7. Dissociation constant (kd) at physiological and acidic pH of engineered antibody HS9 compared to PC9#2_FG.

Antibody	kd (1/s) pH7.4	kd (1/s) pH6.0	ratio kd
PC9#2_FG	6.50E-04	7.40E-03	11.4
HS9	2.04E-04	9.82E-05	0.5

Example 2. Preparation of a Dual Action Fusion Molecule

[0179] A dual action fusion molecule was made according with a large-scale structure as shown in Figure 1A of: GLP-1 moiety—Linker—Antibody Light Chain. SEQ ID NO: 3 was used as the GLP-1 moiety, SEQ ID NO: 4 was used as the linker, and SEQ ID NO: 1 and 2, were used as the heavy and light chain respectively.

[0180] In this embodiment, the N-terminal end of GLP-1 analogue peptides was free in order to most efficiently engage and activate the GLP-1 receptor; peptides were fused to the N-terminal of antibody variable domains. A linker sequence was used in many constructs between the end of the peptide and the start of variable domain in order to minimize the impact of the fusion on peptide and/or antibody activities. Peptides were fused either at the heavy chain or the light chain of antibodies in order to obtain two peptide moieties per fusion molecule. Large scale structure of such fusions are shown Figure 1B (heavy chain fusion) and 1C (light chain fusion). In some embodiments, peptides may be fused at both the heavy and light chains to display four peptide moieties per fusion molecule (shown prophetically in Figure 1D).

[0181] Genes coding for GLP-1 analogue peptides in fusion with antibody variable domains were built by overlapping PCR using standard methods. Unique restriction sites were incorporated at the 5' and 3' end of the DNA fragment to enable cloning in the expression vectors.

[0182] The VH domain, with or without a peptide/linker fusion, was cloned into a vector containing the human heavy chain constant domains and regulatory elements to express whole IgG1 triple mutant (IgG1-TM) heavy chain in mammalian cells. IgG1-TM format is similar to human IgG1 but Fc sequence incorporating mutations L234F, L235E and P331S to reduce its ability to trigger antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity (Oganesyan V. et al., 2008, Acta Cryst., D64: 700–704). The VL domain, with or without peptide/linker fusion, was cloned into a vector for the expression of the human light chain constant domains and regulatory elements to express whole IgG light chain in mammalian cells. An OriP fragment was included in the heavy and light chain expression vectors to facilitate use with CHO cells and to allow episomal replication.

[0183] To obtain IgGs and peptide antibody fusions, the heavy and light chain expressing vectors were transiently transfected into 30mL (small scale) or 400mL (medium scale) of CHO mammalian cells. Table 8 is summarizing the different products that can be obtained by co-transfection of the heavy and light chain expressing vectors with or without a peptide/linker fusion.

Table 8: Product description when using heavy and light chain vectors co-transfection

#	Heavy chain vector	Light chain vector	Product
1	VH **without** peptide fusion	VL **without** peptide fusion	IgG
2	VH **with** peptide fusion	VL **without** peptide fusion	Peptide/antibody VH fusion (Figure 1B)
3	VH **without** peptide fusion	VL **with** peptide fusion	Peptide/antibody VL fusion (Figure 1C)
4	VH **with** peptide fusion	VL **with** peptide fusion	Peptide/antibody VH + VL (shown prophetically in Figure 1D)

[0184] Compounds were expressed and secreted into the medium. Harvests were pooled and filtered before compound purification using Protein A chromatography. Culture supernatants were loaded on a column of appropriate size of MabSelectSure (GE Healthcare Life Sciences) and washed with 1×DPBS (Gibco). Bound compound was eluted from the column using 0.1 M Sodium

Citrate pH 3.0 and neutralised by the addition of Tris-HCl pH 9.0. For small scale transfection, eluted material was buffer exchanged into 1×DPBS using PD10 columns (GE Healthcare). For medium scale transfection, eluted material was further purified by Size Exclusion Chromatography (SEC) using either a HiLoad 16/600 Superdex 200 prep grade column (GE Healthcare) for sample volumes of up to 5ml or a HiLoad 26/600 Superdex 200 column (GE Healthcare) for sample volumes of up to 12ml. Isocratic elution was performed using 1xDPBS as the running buffer.

[0185] Compound concentration was determined spectrophotometrically using an extinction coefficient based on the amino acid sequence according to the protocol in Mach, H. *et al.*, Statistical determination of the average values of the extinction coefficients of tryptophan and tyrosine in native proteins, Anal Biochem, 200(1):74-80 (1992). Purified compounds were analysed for aggregation and degradation using SEC-HPLC and SDS-PAGE. SEC-HPLC was performed by loading 70μL of sample onto a TSKgel G3000SWXL 7.8mmx300mm column (Tosoh Bioscience) using a flow rate of 1mL/min and 0.1M Sodium Phosphate Dibasic anhydrous plus 0.1M Sodium Sulphate at pH 6.8 as running buffer. SDS-PAGE is run by loading 2μg protein on a Nu PAGE® 4%-12% Bis-Tris (Invitrogen) using 1x Nu PAGE® MES SDS Running Buffer (Invitrogen). Compounds from medium scale transfections were further characterized for integrity by Electro-Spray Ionisation Mass Spectrometry (ESI-MS) and tested for endotoxin level using the Limulus Amebocyte Lysate (LAL) Kinetic-QCL (Lonza). For ESI-MS analysis, samples were prepared at 1mg/mL in 10mM Tris-HCl pH 8.0. A 30 minutes reduction at 37°C using 10mM DTT was carried out prior analysis. Data were acquired using a Waters ACQUITY UPLC® I-Class system coupled to a Waters SYNAPT G1 QTOF Mass Spectrometer, operated using MassLynx software. The mobile phases used were highly purified water plus 0.01% trifluoroacidic acid (TFA), 0.1% formic acid (A) and acetonitrile + 0.01% TFA, 0.1% FA (B). Separation between heavy and light chain was achieved using a 2.1 mm × 50 mm Waters BEH300 C4 column, heated at 60°C. The flow rate was set at

0.3 mL/min, with 22 minutes total run time. Samples (10pmol) were injected onto the column and MS data were acquired in positive ion mode, using the following electrospray source parameters: capillary voltage: 3.4kV, 81°C source temperature: 81°C, desolvation temperature: 24°C. Data were acquired between 500 and 4500Da.

Example 3. *In Vitro* Characterization of a GLP-1 Analogue in Genetic Fusion with Anti-PCSK9 Antibodies

[0186] To evaluate the feasibility of generating dual activity molecules by genetic fusion between GLP-1 receptor agonist peptides and anti-PCSK9 antibodies, the GLP-1 analogue peptide of SEQ ID NO:28 was fused using a linker of SEQ ID NO:4 to the heavy or light chain variable domains of seven different previously identified anti-PCSK9 antibodies.

[0187] 1_ PC9#1 with antibody variable heavy chain of SEQ ID NO:10 and antibody variable light chain of SEQ ID NO:11.

[0188] 2_ PC9#2 with antibody variable heavy chain of SEQ ID NO:8 and antibody variable light chain of SEQ ID NO:9.

[0189] 3_ PC9#3 with antibody variable heavy chain of SEQ ID NO: 404 and antibody variable light chain of SEQ ID NO: 405.

[0190] 4_ PC9#4 with antibody variable heavy chain of SEQ ID NO: 406 and antibody variable light chain of SEQ ID NO: 407.

[0191] 5_ PC9#5 with antibody variable heavy chain of SEQ ID NO: 408 and antibody variable light chain of SEQ ID NO: 409.

[0192] 6_ PC9#6 with antibody variable heavy chain of SEQ ID NO: 410 and antibody variable light chain of SEQ ID NO: 411.

[0193] 7_ PC9#7 with antibody variable heavy chain of SEQ ID NO: 412 and antibody variable light chain of SEQ ID NO:413.

[0194] PCSK9 activity of each peptide-antibody fusions was assessed using Homogenous Time Resolved Fluorescence (HTRF) epitope competition assays. In these assays a fluorescence resonance energy transfer (FRET) complex is formed between Streptavidin Cryptate, biotinylated-PCSK9 and a fluorescently (DyLight 650) labelled anti-PCSK9 antibody. Unlabelled peptide-antibody fusions that bind the same or overlapping epitopes of PCSK9 as that bound by the fluorescently labelled antibody will compete resulting in a reduction in FRET signal.

[0195] Labeling of the anti-PCSK9 antibodies was carried out using a DyLight-650 (Thermo Scientific 84536) according to the manufacturer instructions. For the assay all samples and reagents were prepared in assay buffer containing 1X Phosphate Buffered Saline, 0.1% BSA (Sigma A9576) and 0.4M Potassium Fluoride. Test samples of peptide-antibody fusions or control antibodies were prepared in 384 well polypropylene plates by 3-fold serial dilutions. Samples (5µl) or assay buffer (total binding control wells) were transferred to a 384-well assay plate (Costar 3676) and incubated for 4 hours at room temperature with 1nM Streptavidin Cryptate (Cisbio 61SAXLB), 0.05-0.5nM biotinylated-PCSK9 and 0.1-2nM Dy650 labelled anti PCSK9 antibody in a 20µl total assay volume. Non-specific binding (NSB) control wells were set up with the biotinylated PCSK9 omitted. Time resolved fluorescence emission at 665nm and 620nm was measured following excitation at 320nm using the Perkin Elmer Envision. The ratio of the 665nm counts/620nm counts was calculated and multiplied by 10000 to give HTRF Counts. Delta F% was then calculated using the following equation.

$$DeltaF\% = \left(\frac{Sample\,HTRF\,Counts - NSB\,HTRF\,Counts}{NSB\,HTRF\,Counts} \right) \times 100$$

[0196] The results were expressed as % specific binding according to the following equation:

$$\%Specific\,Binding = \left(\frac{(Sample\,DeltaF\% - NSB\,DeltaF\%)}{(Total\,Binding\,DeltaF\% - NSB\,DeltaF\%)} \right) \times 100$$

[0197] Figures 2A-G and Figures 3A-G showing the inhibition of human PCSK9 binding to anti-PCSK9 antibodies using a titration of unlabelled heavy chain or light chain peptide antibody fusions respectively. Isotype match irrelevant antibody NIP228 IgG1-TM alone (Control#1) or in fusion to the heavy (Control#2) or light chain (Control#3) with a GLP-1 analogue peptide of SEQ ID NO:28 using a linker of SEQ ID NO:4 were used as negative controls.

[0198] Potency of each peptide antibody fusion at human GLP-1 receptor was assessed using a cAMP production assay. Stable cell lines expressing human GLP-1 receptor was generated in CHO cells by standard methods. GLP-1 receptor activation by tested compounds will result in downstream production of cAMP second messenger that can be measured in a functional activity assay. Low protein binding 384-well plates (Greiner) were used to perform eleven 1 in 4 serial dilutions of test compound that were made in assay medium (0.1% bovine serum albumin in Hanks Balanced Salt Solution (GIBCO or Sigma), containing 0.5mM IBMX (Sigma)). All sample dilutions were made in duplicate. A frozen cryo-vial of cells expressing human GLP-1 receptor was thawed rapidly in a water-bath, transferred to pre-warmed assay media and spun at 240xg for 5 minutes. Cells were then re-suspended in assay buffer at the optimized concentration of $1x10^5$ cells/mL and dispensed at 5uL per well to black shallow-well u-bottom 384-well plates (Corning). 5 μL of test compound was transferred from the dilution plate to the cell plate and incubated at room temperature for 30 minutes. cAMP levels were measured the cAMP dynamic 2 HTRF kit (Cisbio), following the two step protocol as per manufacturer's recommendations. Briefly, anti-cAMP cryptate (donor fluorophore) and cAMP-d2 (acceptor fluorophore) were made up separately by diluting each 1/20 in conjugate & lysis buffer provided in the kit. 5uL of anti-cAMP cryptate was added to all wells of the assay plate and 5uL of cAMP-d2 added to all wells except non-specific binding wells, to which only conjugate and lysis buffer was added. Plates were incubated at room temperature for one hour and then read on an Envision (Perkin Elmer) using excitation wavelength

of 320nm and emission wavelengths of 620nm and 665nm. Data was transformed to % Delta F as described in manufacturer's guidelines and analyzed by unconstrained 4-parameter logistic fit of data, curve mid-point to determine EC_{50}.

[0199] Activation of the human GLP-1 receptor by heavy chain (A) or light chain (B) peptide antibody fusions are shown in Figures 4A-B, respectively. Free human GLP-1 peptide (Bachem) and irrelevant isotype match NIP228 human IgG1-TM without peptide were used as positive and negative controls respectively.

[0200] To conclude, all tested fusions were able to compete with anti-PCSK9 antibodies for binding to human PCSK9 as well as activating the human GLP-1 receptor. Those fusion molecules between a GLP-1 analogue peptide and different anti-PCSK9 antibodies display dual activity and can be used to provide combined pharmacology.

Example 4. *In vivo* stability of existing GLP-1 analogues in antibody fusion

[0201] Without target-mediated clearance, human IgGs have a long circulating half-life in man of around 21 days. This is notably due to the rescue of internalised antibodies by FcRn receptor. After non-specific uptake by cells, antibodies can bind to FcRn in the acidic environment of endosomes and be directed to the cell surface and back into circulation rather than to lysosomes for degradation.

[0202] In order to achieve maximum efficacy, GLP-1 analogue peptide in fusion with anti-PCSK9 antibody molecules should display adequate *in vivo* activity half-life for both activation of the GLP-1 receptor and PCSK9 suppression mediated by the peptide and the antibody moieties respectively. For instance if the peptide is quickly inactivated after injection, a majority of the product will only be functional for PCSK9 suppression and will not properly engage with the GLP-1 receptor over time to provide efficient glucose control through the dosing period.

[0203] In order to assess *in vivo* stability of existing GLP-1 analogue peptides when in antibody fusion, the following fusions were generated:

[0204] 1: A GLP-1 analogue peptide of SEQ ID NO:28 was fused using a linker of SEQ ID NO:4 to the heavy chain of the irrelevant NIP228 human IgG1-TM antibody of SEQ ID NO: 414 and 415 (compound NIP228_GLP-1_VH).

[0205] 2: Exendin-4 peptide, a GLP-1 analogue derived from Gila monster' saliva, of SEQ ID NO:12 was fused using a linker of SEQ ID NO:4 to the light chain of the anti-PCSK9 antibody PC9#2 of SEQ ID NO 9 (compound PC9#2_Exe4_VL).

[0206] 3: A GLP-1 analogue peptide of SEQ ID NO:28 was fused using a linker of SEQ ID NO:4 to human IgG4 Fc fragment of SEQ ID NO: 416 (compound GLP-1-Fc)

[0207] 4: A GLP-1 analogue peptide of SEQ ID NO:28 was fused using a linker of SEQ ID NO:4 to the light chain of the anti-PCSK9 antibody PC9#2 of SEQ ID NO 9 (compound PC9#2_GLP-1_VL).

[0208] All compounds are active at the human GLP-1 receptor *in vitro* and display EC_{50} in the cAMP assay described in Example 3 of 2.08E-10 M,1.12E-10 M, 1.12E-10 M and 1.03E-10 M for NIP228_GLP-1_VH, PC9#2_Exe4_VL, GLP-1-Fc and PC9#2_GLP-1_VL respectively.

[0209] Compounds NIP228_GLP-1_VH and PC9#2_Exe4_VL were injected intravenously to rat and serum or plasma samples were collected at several timepoints post-injection. Compound concentration in serum or plasma (exposure) and concentration in active compound for GLP-1 activity were determined for each sample. Comparison between decline over time of total compound (exposure) and active compound at GLP-1 receptor provides an assessment of the *in vivo* stability for the GLP-1 analogue peptide in antibody fusion.

[0210] Levels of total human IgG1 antibody in rat plasma or serum were quantified by a generic sandwich enzyme-linked immunosorbent assay (ELISA) method using the Gyrolab platform

(Gyros AB). Human IgG1 was captured by a biotinylated monoclonal anti-human IgG1 antibody (clone JDC-10, Southern Biotech, for plasma samples or in-house clone TM446 for serum samples) at 100ug/mL and detected by an Alexa-labelled monoclonal anti-human IgG1 antibody at 25nM (BD Pharmingen clone G18-145 for plasma samples) or 10 nM (the Binding Site AU003CUS01 for serum samples) on Gyrolab Bioaffy 200 CD. Standards, controls, plasma or serum samples, wash solution, capture and detection antibodies were added to a 0.2 mL 96-well PCR plate (Thermo Scientific) according to the Gyrolab method and loaded onto the machine with the CD200 plate. All samples were analyzed in duplicate. The mean response of each human IgG standard was plotted against concentration and the points were fit using a 5-parameter weighted logistic model using the Gyrolab Evaluator software.

[0211] The concentration of active peptide-antibody fusion at human GLP-1 receptor in rat samples was estimated using an ex-vivo cAMP cell based assay. Reference compounds were spiked into naïve rat serum or plasma at a known concentration to be used as a standard. All samples were serially diluted in assay medium and examined using the cAMP dynamic 2 HTRF kit (Cisbio) as described in Example 3 for serum samples or using the LANCE® Ultra cAMP Detection Kit (Perkin Elmer) for plasma samples.

[0212] Test samples were plotted using the same top concentration as the equivalent reference. The EC_{50} values obtained could then be used to calculate the Sample Ratio (Sample EC_{50} / EC_{50} reference compound) and then the estimated concentration in active GLP-1 compound (known top concentration of reference compound spiked into rat plasma or serum / Sample Ratio). Rat serum or plasma alone has a quenching effect on the cryptate donor signal and gives concentration-dependent activation of cAMP that can be diluted out. Any tested compound must therefore possess cAMP activity above that of the rat serum or plasma baseline (termed the limit of detection) in order to have an observable effect in the activity assay.

[0213] Compound NIP228_GLP-1_VH was injected at 2 mg/kg in three Wistar rats (Charles River) and blood samples for each animal were collected in EDTA tubes containing dPP4 inhibitor at 2 minutes, 1h, 24h, 48h, 120h, 168h and 216h post injection. Total compound and concentration in active GLP-1 compound in rat plasma over time are shown in Figures 5A and 9. Concentration of active compound in samples collected after 48h cannot be determined as they were below the lower limit of quantification of the assay.

[0214] Compound PC9#2_Exe4_VL was injected at 1 mg/kg in three CD rats (Charles River) and blood samples were collected in plain tubes containing dPP4 inhibitor at 30 minutes, 6h, 24h, 48h, 96h, 240h and 336h post injection. Serum samples were prepared by leaving the tubes on the bench for 30 minutes followed by 2 minutes centrifugation at 13000rpm. Exposure and concentration in active GLP-1 compound in rat serum over time are shown in Figure 5B. Concentration of active compound in samples collected after 48h cannot be accurately determined as they were below the lower limit of quantification of the assay.

[0215] *In vivo* half-life in rat of NIP228_GLP-1_VH and PC9#2_Exe4_VL for both exposure and active compound at GLP-1 receptor are presented in Table 9.

Table 9: Compound and active GLP-1 in vivo half-life in rat of existing GLP-1 analogues in antibody fusion

Compound	Experimental design	Compound in vivo half life (h)	Active GLP-1 in vivo half life (h)
NIP228_GLP-1_VH	2mg/kg IV in Wistar rats	63	7.4
PC9_2_Exe4_VL	1mg/kg IV in CD rats	88	5.7

[0216] For both NIP228_GLP-1_VH and PC9#2_Exe4_VL compounds, activity at GLP-1 receptor is loss much quicker than the compound itself demonstrating *in vivo* peptide instability.

[0217] Compounds GLP-1-Fc and PC9#2_GLP-1_VL were injected intravenously to healthy C57/B6 mice (7–8 weeks old, females, Charles River) and plasma samples were collected at

several timepoints. Compound concentration in plasma (exposure) and concentration in active compound for GLP-1 activity were determined for each sample as described above.

[0218] GLP-1-Fc was injected at 1mg/kg. Groups of three mice were sacrificed at each of the following time points: pre-injection, 2 minutes, 1h, 6h, 24h, 48h, 72h and 96h post injection. Blood for each animal were collected in EDTA tubes containing dPP4 inhibitor. Plasma samples were then centrifuged at 14000 rpm for 5 min at 4°C and stored at -80°C pending analysis.

[0219] PC9#2_GLP-1_VL was injected at 5mg/kg and mice were sacrificed at each of the following time points: pre-injection, 5 minutes, 6.5h, 24h, 72h and 168h post injection. Samples were treated as described above.

[0220] Exposure and concentration in active GLP-1 compound in mouse plasma over time for GLP-1-Fc are shown in Figures 5C and 14A and PC9#2_GLP-1_VL are shown in Figures 5D and 10, respectively.

[0221] *In vivo* half-life in mice of GLP-1-Fc and PC9#2_GLP-1_VL for both exposure and active GLP-1 are presented in Table 10.

Table 10: Compound and active GLP-1 in vivo half-life in mice of existing GLP-1 analogues in antibody or Fc fusion

Compound	Experimental design	Compound *in vivo* half life (h)	Active GLP-1 *in vivo* half life (h)
GLP-1-Fc	1mg/kg in C57/B6 mice	~100	16
PC9#2_GLP-1_VL	5mg/kg in C57/B6 mice	~100	18

[0222] As observed for NIP228_GLP-1_VH and PC9#2_Exe4_VL in rat, activity at GLP-1 receptor for both GLP-1-Fc and PC9#2_GLP-1_VL following injection in mice is loss quicker than the compound itself, demonstrating in vivo peptide instability.

[0223] Quick inactivation for both GLP-1 analogues of SEQ ID NO:28 and SEQ ID NO:12 will impact efficient glucose control for PCSK9/GLP-1 fusion molecules and GLP-1 analogue peptides with better *in vivo* stability need to be engineered.

Example 5. Affinity for huPCSK9

[0224] The PCSK9 antibody was used as a benchmark control. Data are presented in Table 11 and a visual representation of the PC9#2_GLP1 molecule and the anti-PC9#2 antibody used as a benchmark control are shown in Figure 8. A visualization of a potential target profile to guide PCSK9 affinity and GLP-1 potency is provided in Figure 6.

[0225] Association (ka or kon), dissociation (kd or koff) and equilibrium dissociation constants (KD) for PCSK9 binding were determined at 25 °C by Surface Plasmon Resonance (SPR) using the Biacore 2000 biosensor (GE Healthcare).

[0226] A Protein G surface was first created on a CM5 sensor chip (GE Healthcare). Human antibodies were then captured on the chip surface before injecting different concentrations of human, cynomolgus or rat PCSK9. Global dissociation rates were first calculated followed by global on-rate calculations both using a 1:1 binding kinetics model.

Table 11: Affinity for huPCSK9 (Biacore)

Test Compound	Kd (nM)	k_{on} ($M^{-1}.s^{-1}$)	k_{off} (s^{-1})
PC9#2 (SEQ ID NO. 8 & 9)	7	7.8E+04	5.5E-04
PC9#2_GLP1 (SEQ ID NO: 8, 9, 4, & 28) (with the light chain fusion SEQ ID NO: 43)	19	2.7E+04	5.0E-04
x change	2.7	2.9	0.9

[0227] This demonstrates that the fusion is only marginally impacting PCSK9 binding.

[0228] Additionally, the dual action fusion molecule HS9_DSB7 was tested in a Biacore assay as described in Example 17 to determine its affinity for human PCSK9. The PCSK9 antibody alone (PC9_2_FG_HS#9 of SEQ ID 1 and 2 for heavy and light chain respectively) was used as a benchmark control. Table 12 provides the data. This data demonstrates that the fusion is only

marginally impacting PCSK9 binding across species (human (Hu), cynomolgus monkeys (Cy) and rat).

Table 12: Affinity for huPCSK9, CyPCSK9, and RatPCSK9 (Biacore)									
Summary	Hu			Cy			Rat		
	ka ($M^{-1}.s^{-1}$)	kd (s^{-1})	KD (M)	ka ($M^{-1}.s^{-1}$)	kd (s^{-1})	KD (M)	ka ($M^{-1}.s^{-1}$)	kd (s^{-1})	KD (M)
PC9_2_FG_HS #9	3.47E+05	3.65E-04	**1.05E-09**	3.19E+05	3.74E-04	**1.17E-09**	9.69E+05	3.45E-04	**3.56E-10**
HS9_DSB7	1.46E+05	2.87E-04	**1.97E-09**	9.49E+04	2.84E-04	**2.99E-09**	5.07E+05	2.67E-04	**5.27E-10**

Example 6. GLP-1 Potency in cAMP Cell-Based Assay

[0229] PC9#2_GLP-1 was tested in a cAMP cell-based assay to determine its potency as described in Example 3. The GLP1 peptide alone was used as a control and GLP-1Fc was used as a benchmark. Data are presented in Table 13 and a visual representation of the PC9#2_GLP1 molecule and the GLP-1 Fc used as a benchmark control are shown in Figure 8.

Table 13: GLP-1 Potency in cAMP Cell-Based Assay		
Test Compound	**EC50 (pm)**	**x change**
PC9#2_GLP1 (SEQ ID NO: 8, 9, 4, & 28)	290	2.4
GLP1-Fc (	120	1
GLP1 peptide (SEQ ID NO: 29)	15	0.1

[0230] This data demonstrates that there was no significant loss of GLP-1 potency compared to the benchmark molecule GLP-1-Fc following the fusion of the GLP-1 analogue peptide to the light chain of the anti-PCSK9 antibody PC9#2.

Example 7. Dulaglutide: A Benchmark Molecule

[0231] Figure 14A provides stability in rat of GLP-1-Fc benchmark. Benchmark molecule is a GLP-1 moiety fusion to an Fc portion of an antibody, as shown in Figure 14A.

[0232] Compounds GLP-1-Fc was injected intravenously to healthy C57/B6 mice (7–8 weeks old, females, Charles River) and plasma samples were collected at several timepoints.

Compound concentration in plasma (exposure) and concentration in active compound for GLP-1 activity were determined for each sample as described in Example 4.

[0233] GLP-1-Fc was injected at 1mg/kg. Groups of three mice were sacrificed at each of the following time points: pre-injection, 2 minutes, 1h, 6h, 24h, 48h, 72h and 96h post injection. Blood for each animal were collected in EDTA tubes containing dPP4 inhibitor. Plasma samples were then centrifuged at 14000 rpm for 5 min at 4°C and stored at -80°C pending analysis.

[0234] GLP-1 activity is lost at a quicker rate than the compound, demonstrating peptide instability. The line with the squares corresponds to the serum concentration of GLP-1-Fc and the line with the circles corresponds to the activity of GLP-1-Fc for the same samples. See Figure 14A.

Example 8. Fusion Molecules with Enhanced *In Vivo* Stability Profiles

A) Evaluating GLP-1 Analogue Peptides in Antibody Fusion with Enhanced *In Vivo* Stability Profiles

[0235] To improve *in vivo* peptide stability of antibody fusion molecules, steric hindrance was engineered around the peptide to protect it from degradation. This was done either by introducing a bulky sugar motif or by engineering an inter molecular disulphide bridge.

[0236] It has been demonstrated that addition of N-glycosylation consensus motifs can increase *in vivo* stability and duration of action of proteins (Elliott S. *et al.*, Nat. Biotech., 2003, **21**, 414–421). GLP-1 analogue peptides incorporating an extra N-glycosylation motif, NxS or NxT where x can be any amino acid except proline, at the C-terminus of the peptide or in the linker between the peptide and the antibody have been engineered in fusion with the light chain of anti-PCSK9 antibody PC9#2 (antibody light chain, SEQ ID NO: 9). Peptide and linker amino acid sequence for eight of those compounds (named NGS for N-Glysosylation Site) are shown in Figure 7A. Amino acid changes to generate the glycosylation motif are shown in bold underlined.

[0237] Among those eight compounds, only PC9_2_GLP-1_NGS#7 shows a high glycosylation yield by SDS-PAGE. This was detected by an increase in the molecular weight of the

light chain compared to the control compound without the glycosylation consensus sequence, and with no visible lower molecular band corresponding to the non-glycosylated light chain product. Glycosylation for PC9_2_GLP-1_NGS#7 was further confirmed by ESI mass spectrometry.

[0238] It has also been shown that introducing an inter-disulphide bond could be a successful approach to improve *in vivo* stability of GLP-1 analogues as free peptide (Li Y. *et al.*, Peptides, 2011, 21, 1303–1312).

[0239] Exendin-4 peptide variants incorporating two cysteine residues to form the disulphide bridge as well as, if appropriate, a glycine C-terminus cap in order to facilitate the bonding were fused to the light chain of anti-PCSK9 antibody PC9#2 (SEQ ID NO: 9). Peptide amino acid sequence for the three compounds initially generated (named DSB for DiSulphide Bridge) are shown in Figure 7B (as SEQ ID NOs: 30-32). Cysteine residues are shown in black, other mutated residues are shown as underline and additional glycine residues at the C-terminus cap are shown in grey.

[0240] For DSB#1 variant, the first cysteine was engineered in position 9 instead of an aspartic acid and using a C-terminus cap, incorporating the second cysteine, of sequence: GGGGGGGGGGGCGG (SEQ ID NO: 401).

[0241] For DSB#2 variant, the first cysteine was engineered in position 4 instead of a glycine and using a C-terminus cap, incorporating the second cysteine, of sequence: GGGGGGGGGGGCG (SEQ ID NO: 402).

[0242] For DSB#3 variant, the first cysteine was engineered in position 18 instead of an alanine but no C-terminus cap was used. The second cysteine was introduced at position 39 of the Exendin-4 sequence instead of a serine. Proline 38 was also changed into a glycine to generate more flexibility in the tryptophan cage of Exendin-4 in order to facilitate the formation of the disulphide bridge.

[0243] PC9_2_Exe4_DSB#2 in light chain fusion does not express significantly in mammalian cells and was not further characterised but sufficient amount of PC9_2_Exe4_DSB#1 and PC9_2_Exe4_DSB#3 were obtained. Integrity and identity of the fusions were confirmed by ESI mass spectrometry before *in vivo* experiments.

[0244] *In vivo* stability of PC9_2_GLP-1_NGS#7, PC9_2_Exe4_DSB#1 and PC9_2_Exe4_DSB#3 was assessed in mouse by following both compound exposure and concentration in active GLP-1 over time as described in Example 4.

[0245] Healthy C57/B6 mice (7–8 weeks old, females, Charles River) received one single intravenous (IV) dose of PC9_2_GLP-1_NGS#7 at 40mg/kg, PC9_2_Exe4_DSB#1 at 10.8mg/kg or PC9_2_Exe4_DSB#3 at 5mg/kg. Groups of three mice were sacrificed at each of the following time points: pre-injection, 5 minutes, 6h, 24h, 72h and 168 h post injection and blood for each animal were collected in EDTA tubes containing dPP4 inhibitor. Plasma samples were then centrifuged at 14000 rpm for 5 min at 4°C and stored at -80°C pending analysis.

[0246] All three compounds are active at the human GLP-1 receptor *in vitro* but display different EC_{50} in the cAMP assay: 1.94^{-8} M, 5.25^{-9} M and 3.25^{-10} M for PC9_2_GLP-1_NGS#7, PC9_2_Exe4_DSB#1 and PC9_2_Exe4_DSB#3 respectively. Doses were adjusted as much as possible based on potency to generate a signal above the lower limit of quantification in the cAMP ex-vivo assay in order to calculate concentration in active GLP-1 compound over a significant period of time.

[0247] Exposure and concentration in active GLP-1 compound in mouse plasma over time for PC9_2_GLP-1_NGS#7 are shown in Figures 12 and 13A, PC9_2_Exe4_DSB#1 are shown in Figures 13B and 14B, and PC9_2_Exe4_DSB#3 fusion molecules are shown in Figures 11 and 13C, respectively.

[0248] *In vivo* half-life of PC9_2_GLP-1_NGS#7, PC9_2_Exe4_DSB#1 and PC9_2_Exe4_DSB#3 for both exposure and active GLP-1 are presented in Table 14.

Table 14: Compound and active GLP-1 *in vivo* half-life of engineered GLP-1 analogues in antibody fusion

Compound	Experimental design	Compound *in vivo* half life (h)	Active GLP-1 *in vivo* half life (h)
PC9_2_GLP-1_NGS#7	40 mg/kg IV in C57/B6 mice	~100	76
PC9_2_Exe4_DSB#1	10.8 mg/kg IV in C57/B6 mice	~100	~100
PC9_2_Exe4_DSB#3	5 mg/kg IV in C57/B6 mice	~100	36

[0249] Compared to parent molecules NIP228_GLP-1_VH and PC9#2_Exe4_VL (Table 9), all three compounds have improved *in vivo* stability for GLP-1 activity with half-life of 76h, around 100h and 36h for PC9_2_GLP-1_NGS#7, PC9_2_Exe4_DSB#1 and PC9_2_Exe4_DSB#3 respectively.

[0250] Quite interestingly, PC9_2_Exe4_DSB#1 appears fully stable in mice for up to 7 days with no observed loss of GLP-1 activity when compared to compound exposure (Figure 13B).

[0251] Such data are suggesting that generating steric hindrance around GLP-1 analogues can increase *in vivo* activity half-life of the peptide in antibody fusion.

B) Evaluating Fusion Molecules with Enhanced *In Vivo* Stability Profiles

[0252] The protocol discussed in Example 4 and 8A was followed to administer various compounds to mice and to plot the concentration of the compounds in the plasma over time. Compound potency (EC50) at human GLP-1 receptor was determined using the cAMP assay as described in Example 3.

[0253] Fusion molecule optimization was guided through an *in vivo* PK/stability assessment. As shown in Figures 14A, 11 and 12, peptide engineering produced fusion molecules with enhanced *in vivo* stability profiles. Benefits were seen with disulfide bridge stabilization for which there was

longer retention of activity with minimum impact on potency at GLP-1 receptor. NGS#7 also has an improved stability profile but a low potency (19 nM) compared to DSB#3 (340 pM).

[0254] Figure 14A shows a GLP-1-Fc benchmark molecule with an EC_{50} of 100 pm.

[0255] Figure 11 shows a disulfide bridged variant (PC9_2_DSB#3) (SEQ ID NO: 49 and SEQ ID NO: 8) with an EC_{50} of 340 pM.

[0256] Figure 12 shows an n-glycosylation variant (PC9_2_NGS#7) (SEQ ID NO: 50 and SEQ ID NO: 8) with an EC_{50} of 19 nM.

[0257] *In vivo* half-life for those compounds are presented in Table 15.

Table 15: Compound and active GLP-1 *in vivo* half-life of engineered GLP-1 analogues in antibody fusion

Compound	Experimental design	Compound *in vivo* half life (h)	Active GLP-1 *in vivo* half life (h)
GLP-1-Fc	1mg/kg in C57/B6 mice	~100	16
PC9_2_Exe4_DSB#3	5 mg/kg IV in C57/B6 mice	~100	36
PC9_2_GLP-1_NGS#7	40 mg/kg IV in C57/B6 mice	~100	76

Example 9. A PCSK9/GLP-1 Fusion Demonstrates an Ideal Stability/Activity Profile

[0258] The dual action fusion molecule was evaluated for Fc-exposure and GLP-1 activity in a mouse model. Dosing was 10.8 mg/kg intravenously and the *in vitro* potency was 5200 pM. Protocol was as described in Example 8. The data were compared to the GLP-1 Fc benchmark molecule, with dosing of 1 mg/kg intravenously and an *in vitro* potency of 100 pM. Similar protocol was here used as described in Example 4. Results are shown in Figures 14A (dulaglutide) and 14B (PCSK9/GLP-1 fusion PC9_2_DSB#1) (SEQ ID NO: 48 and SEQ ID NO: 8). There was no loss of GLP-1 activity in the mouse for up to 7 days.

Example 10. Production and Purification of Early GLP-1 Analogue Peptide Antibody Fusions with an Intramolecular Disulphide Bridge

[0259] Quality of the material post-protein A purification from medium scale batches was poor for PC9_2_Exe4_DSB#1 and PC9_2_Exe4_DSB#3 as lot of aggregates were detected by

SEC-HPLC. Preparative Size Exclusion Chromatography using Superdex 200 prep grade columns was then used to further purify the compounds as described in Example 2.

[0260] Preparative SEC chromatograms for PC9_2_Exe4_DSB#1 and PC9_2_Exe4_DSB#3 are shown in Figures 15A and 15B, respectively. A significant proportion (25-40%) of the material is aggregated as shown by additional peaks at early retention time. Fractions containing the monomeric compound were collected to obtain the material used for *in vivo* testing. See Example 8 and 9.

[0261] Scalability of PC9_2_Exe4_DSB#1 production was further assessed by transiently transfected 48.2L of CHO mammalian cells in wavebags (GE Healthcare). Compound was purified by using a Protein A column followed by two additional purification steps using a mixed-mode resin.

[0262] A high level of aggregation (>25%) was detected in the harvest and efficient purification of the monomer product was particularly challenging. Three chromatography steps were required to generate a product at 95.9% purity by SEC-HPLC. This was very detrimental to the purification yield with an overall recovery of around 3.4%. In addition, the titre in harvest was at 104mg/L which is low compared to monoclonal antibodies using a similar expression system. Production of DSB#1 disulphide bridge GLP-1 analogue in fusion with the light chain of anti-PCSK9 antibody HS9 (SEQ ID NO: 2) gave very similar results.

Example 11. Aggregation

[0263] The dual action fusion molecule (PCSK9/GLP-1 fusion PC9_2_DSB#1) (SEQ ID NO: 48) has the potential for aggregation and in some embodiments monomer is desired to be selected. Aggregates, detected during an analytical SEC-HPLC following protein A purification as described in Example 2, are shown in Figure 16. Thus, in some embodiments additional engineering may be desired to improve the monomeric profile.

Example 12. GLP-1 Analogue Peptides in Antibody Fusion with Enhanced Monomeric Profiles

[0264] Additional Exendin-4 disulphide bridge peptides (DSB) in fusion with the light chain of PC9#2 (SEQ ID NO: 9) were generated in order to improve the monomeric profile during production of the peptide antibody molecule. Different positions of the cysteine bridge as well as length of the glycine rich C-terminus cap and various glycine point mutations in the C-terminus of the Exendin-4 peptide were engineered in order to facilitate the formation of the disulphide bond and ultimately reduce aggregation during production probably due to disulphide scrambling. Peptide engineering work was guided using the 3-D NMR structure of Exendin-4 (Neidigh, JW *et al.*, Biochemistry, 2001, 40, 13188-200.).

[0265] A total of ten DSB peptide anti-PCSK9 fusions were produced at small scale and screened for an improved monomeric profile by SEC-HPLC. Peptide sequences are described in Figure 17. PC9_2_Exe4_DSB#4 did not significantly express and was not further characterised.

[0266] Percentages of aggregate for the nine fusions compared to PC9_2_Exe4_VL and PC9_2_Exe4_DSB#1 determined by analytical SEC-HPLC after protein A purification are described in Table 16.

Table 16. Percentage of aggregate post protein A purification for disulphide bridge exendin-4 variants in fusion with the light chain of PCSK9 antibody PC9_2

#	Compound	% aggregate by analytical SEC
1	PC9_2_Exe4_VL	2.9
2	PC9_2_Exe4_DSB#1	26.5
3	PC9_2_Exe4_DSB#5	20.5
4	PC9_2_Exe4_DSB#6	23.3
5	PC9_2_Exe4_DSB#7	5.1
6	PC9_2_Exe4_DSB#8	51.1
7	PC9_2_Exe4_DSB#9	7.8
8	PC9_2_Exe4_DSB#10	7.3
9	PC9_2_Exe4_DSB#11	7.6
10	PC9_2_Exe4_DSB#12	20.1

Table 16. Percentage of aggregate post protein A purification for disulphide bridge exendin-4 variants in fusion with the light chain of PCSK9 antibody PC9_2		
#	Compound	% aggregate by analytical SEC
11	PC9_2_Exe4_DSB#13	17.1

[0267] Four compounds among the nine tested achieve a percentage aggregate below 10%: PC9_2_Exe4_DSB#7, PC9_2_Exe4_DSB#9, PC9_2_Exe4_DSB#10 and PC9_2_Exe4_DSB#11. PC9_2_Exe4_DSB#7 has the lowest percentage aggregate at 5.1% compared to 26.5% for the early fusion PC9_2_Exe4_DSB#1. Exendin-4 antibody fusion without a disulphide bridge, PC9_2_Exe4_VL, shows 2.9% aggregate.

[0268] Productions of PC9_2_Exe4_DSB#7 and PC9_2_Exe4_DSB#9 were scaled up to supply material in sufficient quantity to perform *in vivo* stability experiments. Figures 18 and 19 show showing preparative SEC chromatograms of PC9_2_Exe4_DSB#7 and PC9_2_Exe4_DSB#9 respectively following an initial protein A purification as described in Example 2. Unlike PC9_2_Exe4_DSB#1 and PC9_2_Exe4_DSB#3 (Figures 15A and B), no significant proportion of aggregates was detected during that purification step.

[0269] In order to check that the optimised DSB peptide antibody fusions with an improved monomeric profile do exhibit a superior *in vivo* stability compared to Exendin-4 antibody fusion, PC9_2_Exe4_DSB#7 and PC9_2_Exe4_DSB#9 were injected intravenously in three CD rats for each compound at 10mg/kg and 1mg/kg respectively. Serum samples for each animal were collected at 30 minutes, 6h, 24h, 48h, 96h, 240h and 336h post injection. Compound exposure and concentration in active GLP-1 were measured over time as described in Example 4.

[0270] Both compounds are active at the human GLP-1 receptor in the cAMP assay with EC_{50} of 9.45E-10 M for PC9_2_Exe4_DSB#7 and 1.24E-10 M for PC9_2_Exe4_DSB#9. PC9_2_Exe4_DSB#7 is around eight time less potent than PC9_2_Exe4_DSB#9 and as thus been

injected at ten time higher a dose to generate a signal above the lower limit of quantification in the cAMP ex-vivo assay over a significant period of time.

[0271] Exposure and concentration in active GLP-1 compound in rat serum over time for PC9_2_Exe4_DSB#7 and PC9_2_Exe4_DSB#9 fusion molecules are shown in Figures 20 and 21, respectively. Concentrations in active GLP-1 were normalised to the exposure at the first time point (30min) to simplify the analysis.

[0272] PC9_2_Exe4_DSB#7 and PC9_2_Exe4_DSB#9 have a half-life for GLP-1 activity of 44.2h and 12.5h respectively compared to 5.7h for the parent Exendin-4 fusion molecule PC9_2_Exe4_VL (see Table 9)). Concentrations of active compound for PC9_2_Exe4_DSB#9 samples collected after 96h cannot be determined as they were below the lower limit of quantification of the assay.

[0273] Those data are demonstrating that both PC9_2_Exe4_DSB#7 and PC9_2_Exe4_DSB#9 have an improved *in vivo* activity half-life of the GLP-1 analogue peptide when in antibody fusion compared to the parent fusion molecule.

[0274] As described above peptide engineering can manage the aggregation profile. Mutations were made in the position of the cysteine bridge in the peptide and the composition and length of the peptide/C terminus of the GLP-1 moiety.

[0275] The protocol was as described above.

[0276] PCSK9/GLP-1 fusion (PC9_2_DSB#7) (SEQ ID NO: 51 and SEQ ID NO: 8) achieved >90% monomer by SEC-HLPC in small-scale batch. Aggregation was still detected in medium scale batch but to a much lower extended than PCSK9/GLP-1 fusion PC9_2_DSB#1 (SEQ ID NO: 48 and SEQ ID NO: 8).

[0277] Results are presented in Figures 15A, 18, 23 and Table 16.

Example 13. Pharmacokinetics and pharmacodynamics modeling of GLP-1 Analogue Peptides in Fusion with Anti-PCSK9 Antibodies

[0278] To guide the design of PCSK9/GLP-1 fusion molecules, a pharmacokinetic (PK) - pharmacodynamic (PD) model has been developed using prior data on the relationship between PCSK9 suppression and affinity of anti-PCSK9 antibodies tested in the clinic as well as data on the approved GLP-1 receptor agonist molecules Liraglutide and Dulaglutide.

[0279] The potency of GLP-1 analogue peptide in fusion with anti-PCSK9 antibody was scanned to identify the optimum range that would result in comparable GLP-1 activity to marketed drugs using a dose able to generate sufficient PCSK9 suppression. Simulations were performed using the pharmacokinetics, plasma protein binding, and receptor affinity properties of Dulaglutide to obtain the potency-normalised GLP-1 activity over time of that compound. For PCSK9/GLP-1 fusion molecule, the PK properties are assumed to be those of a typical human antibody directed towards PCSK9 and the information was derived from compounds in the clinic. These simulations indicate that a 60 mg subcutaneous weekly dose of PCSK9/GLP-1 fusions with an affinity of 3.9nM for human PCSK9 should result in greater than 90% PCSK9 suppression over the dosing period at steady-state (Figure 22A).

[0280] Using that dosing regimen, simulations indicate that potency of PCSK9/GLP-1 fusion molecules at human GLP-1 receptor should be within 3-5 nM in order to achieve similar GLP-1 activity compared to existing molecules (Figure 22B). Potency of Dulaglutide in those simulations was set-up at 80 pM suggesting that potency of PCSK9/GLP1 fusion molecules need to be around 30 to 60 fold lower than Dulaglutide in order to manage nausea side effect associated with GLP-1 receptor agonist molecules at the dose required to efficiently suppress PCSK9.

Example 14. GLP-1 analogue peptides with reduced potency at the human GLP-1 receptor

[0281] Methods for reducing potency of the GLP-1 peptide or GLP-1 analogues are well known in the art as for instance mutating or introducing non-natural amino acids at key residues in

the peptide. Critical residues for binding of GLP-1 peptide to the receptor and activity were notably identified by alanine scanning (Adelhorst, K. *et al.*, J. Bio. Chem., 1994, 269, 6276-6278).

[0282] To further demonstrate the feasibility to reduce potency at GLP-1 receptor, a panel of GLP-1 analogues with a mutation or non-natural amino acids in position 2 or 3, using the 7-36 GLP-1 sequence, were synthetized as free peptide and tested for activity at the human GLP-1 receptor in the cAMP *in vitro* assay as described in Example 3. Data are summarised in Table 17 below. Aib is for 2-aminoisobutyric acid and Orn for ornithine.

Table 17. Potency at human GLP-1R in the cAMP assay of GLP-1 point mutant peptides				
#	Peptide	Modification	Potency at huGLP-1R in cAMP assay (nM)	Fold difference in EC50 vs GLP-1
1	GLP-1	/	0.10	/
2	g769	E3D	0.10	1
3	g766	A2Aib	0.11	1.1
4	g770	A2P	0.15	1.5
5	g767	E3Q	0.28	2.8
6	g762	A2S	0.29	2.9
7	g755	A2H	0.32	3.2
8	g752	A2V	0.56	5.6
9	g749	A2G	0.63	6.3
10	g768	E3N	0.68	6.8
11	g751	A2I	1.78	17.8
12	g765	A2Q	2.98	29.8
13	g763	A2T	4.30	43
14	g753	A2F	4.30	43
15	g756	A2W	5.46	54.6
16	g754	A2Y	14.4	144
17	g764	A2N	21.0	210
18	g750	A2L	80.0	800
19	g761	A2Orn	86.4	864
20	g757	A2E	103.3	1033
21	g758	A2D	>100	>1000
22	g760	A2R	>80	>800
23	g759	A2K	>100	>1000

[0283] Those data demonstrate that EC_{50} of GLP-1 analogue peptides can be modulated to achieve a defined potency reduction.

Example 15. GLP-1 Analogue Peptides in Antibody Fusion with Reduced Potency at the Human GLP-1 Receptor

To reduce the potency of DSB#7 GLP-1 analogue peptide (SEQ ID NO: 13) in fusion with the light chain of the anti-PCSK9 antibody HS9 (SEQ ID NO: 2), point mutations were introduced at position G2, E15, V19, I23 or L26 in the peptide using standard molecular biology techniques. A total of 28 mutants of the peptide antibody fusion molecule were

produced and tested for activity at the human GLP-1 receptor using the cAMP assay as described in Example 3. List of the compounds and activity data are presented in Table 18.

Table 18. Potency at human GLP-1R and percentage maximum activation compared to GLP-1 of DSB#7 point mutants in light chain fusion of PCSK9 antibody HS9

#	Compound	Potency at huGLP-1R in cAMP assay (M)	% Max Effect	Fold difference in EC50 vs GLP1-Fc	SEQ ID NO:
1	HS9_DSB7_G2Y	1.7E-08	10	603	444
2	HS9_DSB7_G2V	1.2E-09	93	43	445
3	HS9_DSB7_G2T	6.3E-09	73	227	446
4	HS9_DSB7_G2Q	1.2E-08	86	436	447
5	HS9_DSB7_G2N	1.9E-08	31	685	448
6	HS9_DSB7_G2I	2.3E-09	87	81	449
7	HS9_DSB7_G2F	5.8E-09	69	209	450
8	HS9_DSB7_E15G	6.8E-09	91	243	451
9	HS9_DSB7_E15A	1.2E-09	92	42	452
10	HS9_DSB7_V19T	4.0E-10	95	14	453
11	HS9_DSB7_V19S	2.7E-09	94	95	454
12	HS9_DSB7_V19G	1.3E-08	93	479	455
13	HS9_DSB7_V19A	1.3E-09	96	46	456
14	HS9_DSB7_I23T	Inactive	Inactive	/	457
15	HS9_DSB7_I23S	Inactive	Inactive	/	458
16	HS9_DSB7_I23G	5.6E-07	82	2.0E+04	459
17	HS9_DSB7_I23A	6.1E-07	67	2.2E+04	460
18	HS9_DSB7_L26T	1.3E-07	87	4.7E+03	461
19	HS9_DSB7_L26S	Inactive	Inactive	/	462
20	HS9_DSB7_L26P	1.4E-06	60	5.1E+04	463
21	HS9_DSB7_L26N	4.3E-06	44	1.5E+05	464
22	HS9_DSB7_L26Q	5.9E-06	34	2.1E+05	465
23	HS9_DSB7_L26M	5.3E-08	90	1.9E+03	466
24	HS9_DSB7_L26I	7.4E-10	96	27	467
25	HS9_DSB7_L26H	8.0E-08	87	2.9E+03	468
26	HS9_DSB7_L26G	2.8E-06	81	1.0E+05	469
27	HS9_DSB7_L26E	Inactive	Inactive	/	470
28	HS9_DSB7_L26D	Inactive	Inactive	/	471
29	HS9_DSB7	2.1E-10	96	8	472
30	GLP1	1.9E-11	100	0.7	
31	GLP1-Fc	2.8E-11	99	1	

[0284] Five out of the twenty-eight constructs are inactive. The others compounds display very diverse potency at human GLP1 receptor ranging from 400pM for HS9_DSB7_V19T to almost 6uM for HS9_DSB7_L26Q. In addition some compounds, as HS9_DSB7_G2Y or HS9_DSB7_L26Q, are partial agonists: they do not provide the same level of activation at saturating dose compared to GLP-1 peptide.

[0285] Based on PKPD modeling in Example 13 the dual activity anti-PCSK9 antibody GLP-1 receptor agonist molecules need to have a potency reduction at human GLP-1 receptor compared to the GLP-1-Fc benchmark of around 30 to 60 fold in order to manage nausea at the dose required to efficiently suppress PCSK9 antigen.

[0286] Four peptide antibody fusions (HS9_DSB7_G2V, HS9_DSB7_E15A, HS9_DSB7_V19A and HS9_DSB7_L26I) display a potency between 700pM and 1.4nM corresponding to a 25 to 50 fold loss compared to the benchmark GLP1-Fc. All those four compounds are full agonists at human GLP-1 receptor with a percentage of maximum activation greater than 90% compared to the GLP-1 peptide.

Example 16. Characterisation of GLP-1 analogue peptides in fusion with anti-PCSK9 antibody

A) Developability Assessment of GLP-1 Analogue Peptides in Fusion with Anti-PCSK9 Antibody

[0287] The four selected peptide antibody fusions at the desired human GLP-1R potency (HS9_DSB7_G2V, HS9_DSB7_E15A, HS9_DSB7_V19A and HS9_DSB7_L26I) were produced in large scale to support further characterisation. To assess propensity of the compounds to aggregate during production, post protein A purification samples were tested by analytical SEC-HPLC. Data are summarised in Table 19.

Table 19. SEC-HPLC analysis of peptide/antibody lead molecules following Protein A purification				
#	Compound	% Aggregate	% Monomer	% Truncate
1	HS9_DSB7_G2V	25.08	74.64	0.28
2	HS9_DSB7_E15A	21.04	78.6	0.36
3	HS9_DSB7_V19A	4.33	95.43	0.24
4	HS9_DSB7_L26I	5.96	93.81	0.23

[0288] Only two out of the four fusions (HS9_DSB7_V19A and HS9_DSB7_L26I) achieve a percentage monomer greater than 90% post protein A purification. HS9_DSB7_V19A presents the best profile with more than 95% monomer. HS9_DSB7_G2V and HS9_DSB7_E15A are significantly prone to aggregation during production with percentage aggregate greater than 20% compared to less than 5% for HS9_DSB7_V19A.

[0289] Purified compounds were concentrated using centrifugal spin concentrators with a molecular weight cut-off of 30kDa to achieve a target concentration of 50mg/mL in default formulation buffer. Concentration of HS9_DSB7_G2V and HS9_DSB7_E15A was stopped at 38.7 and 33.7 mg/mL respectively as it was noticed that further volume reduction leads to a drop in protein concentration. No such issue was observed during the concentration step of HS9_DSB7_V19A and HS9_DSB7_L26I.

[0290] Samples were then incubated at 5°C or 40°C for 4 weeks followed by analytical SEC-HPLC in order to assess storage stability. Results are summarized in Table 20.

Table 20. Purity and aggregation parameters for purified peptide/antibody lead molecules after 4 weeks incubation at 5°C or 40°C						
#	Compound	[C] mg/mL	Purity after 4 weeks at 5°C (%)	Purity after 4 weeks at 40°C (%)	Aggregation rate per month at 5°C (%)	Aggregation rate per month at 40°C (%)
1	HS9_DSB7_G2V	38.7 *	88.6	77.9	1.4	9.2
2	HS9_DSB7_E15A	33.7 *	93.9	80.8	0.81	13.2
3	HS9_DSB7_V19A	52	98.7	90.1	0.3	6.3
4	HS9_DSB7_L26I	47.2	97	87.6	0.32	7.1

[0291] Both HS9_DSB7_V19A and HS9_DSB7_L26I display better stability parameters than HS9_DSB7_G2V and HS9_DSB7_E15A. For instance, the first two have an aggregation rate per month at 5°C of around 0.3% compared to 0.8 and 1.4% for HS9_DSB7_E15A and HS9_DSB7_G2V respectively.

B) Single intravenous dose pharmacokinetics in rat of GLP-1 analogue peptides in fusion with anti-PCSK9 antibody

[0292] Pharmacokinetic profile of the four selected peptide antibody fusions at the desired human GLP-1R potency was assessed as described in Example 4 following a single intravenous bolus in three CD rats at 60, 53, 58.5 and 60 mg/kg for HS9_DSB7_G2V, HS9_DSB7_E15A, HS9_DSB7_V19A and HS9_DSB7_L26I respectively. A high dose of the compound (above 50mg/kg) was used to saturate the PCSK9 sink for a significant period of time to determine PK parameters during the linear phase, without any target mediated drug disposition component.

[0293] Blood samples were collected at 30 minutes, 6h, 24h, 48h, 96h, 168h, 240h and 336h post injection. Concentrations for the four compounds over time are shown in Figure 24 and half-life data are summarized in Table 21.

Table 21. In vivo half-life of peptide/antibody lead molecules in rats after a single i.v. injection.

#	Compound	Design	Exposure half-life (h)
1	HS9_DSB7_G2V	60 mg/kg iv in CD rats	48
2	HS9_DSB7_E15A	53 mg/kg iv in CD rats	41
3	HS9_DSB7_V19A	58.5 mg/kg iv in CD rats	128
4	HS9_DSB7_L26I	60 mg/kg iv in CD rats	39

[0294] The four fusions molecules have significant different profiles despite being very close in sequence and sharing the same antibody backbone. HS9_DSB7_V19A has the longest *in vivo* half-life of the four fusions, 128h compared to for instance only 39h for HS9_DSB7_L26I.

Example 17. Affinity and Kinetic Parameters Determination for PCSK9 Across Species of GLP-1 Analogue Peptide in Fusion with Anti-PCSK9 Antibody

[0295] Association (ka), dissociation (kd) and equilibrium dissociation constants (KD) for human, cynomolgus and rat PCSK9 binding to the GLP-1 analogue anti-PCSK9 fusion HS9_DSB7_V19A human IgG1-TM were determined at 25 °C by Surface Plasmon Resonance (SPR) using the Biacore 2000 biosensor (GE Healthcare), essentially as described by Karlsson et al. (J. Immunol. Methods (1991), vol. 145, p. Dear229-40). Anti-PCSK9 antibody PC9#3 human IgG1-TM was used as benchmark (Variable heavy chain of SEQ ID NO: 404 and variable light chain of SEQ ID NO: 405.

[0296] A mouse anti-human IgG monoclonal antibody surface was first created using a Human Antibody Capture Kit and CM5 sensor chip (GE Healthcare). Human antibody compounds were captured at a flow rate of 10 μL/minute for 3 minutes. Recombinant human Avi_PCSK9_Flag_His (in-house), cynomolgus Avi_PCSK9_Flag_His (in-house) and His-tagged rat PCSK9 (SinoBiological) were diluted to concentrations ranging from 1 nM to 200 nM in running buffer (10 mM sodium phosphate pH 7.4, 150 mM sodium chloride, 1 mg/mL BSA, 0.05% Tween20) and injected over the chip surface for 10 minutes, followed by running buffer only for a 10 minutes dissociation phase. The surface of the chip was regenerated using 3 M magnesium chloride between each antibody application. Global dissociation rates were first calculated followed by global on-rate calculations both using a 1:1 binding kinetics model.

[0297] Results are shown in Table 22.

Table 22: Kinetic parameters determined by Biacore of peptide/antibody lead molecule HS9_DSB7_V19A for human, cynomolgus and rat PCSK9 compared to anti-PCSK9 antibody PC9#3

Human PCSK9			
kinetic parameters	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**
PC9#3	6.4E+05	4.4E-04	7.0E-10
HS9_DSB7_V19A	1.9E+05	1.1E-04	6.0E-10

Table 22: Kinetic parameters determined by Biacore of peptide/antibody lead molecule HS9_DSB7_V19A for human, cynomolgus and rat PCSK9 compared to anti-PCSK9 antibody PC9#3			
Human PCSK9			
Cynomolgus PCSK9			
kinetic parameters	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**
PC9#3	4.2E+05	4.2E-04	1.0E-09
HS9_DSB7_V19A	1.1E+05	1.9E-04	1.7E-09
Rat PCSK9			
kinetic parameters	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**
PC9#3	9.0E+05	4.7E-03	5.2E-09
HS9_DSB7_V19A	3.7E+05	2.1E-04	5.7E-10

[0298] Fusion molecule HS9_DSB7_V19A has an affinity at pH7.4 for human PCSK9 of 600 pM very similar to the benchmark anti-PCSK9 antibody PC9#3. Interestingly HS9_DSB7_V19A, with a four time lower dissociation constant, is less prone to dissociate from human PCSK9 compared to PC9#3.

[0299] In addition, HS9_DSB7_V19A can strongly bind to cynomolgus and rat PCSK9 at physiological pH with equilibrium dissociation constants close to the human PCSK9 value (1.7nM and 570pM respectively).

Example 18. Specificity for PCSK9 compared to closely related human proteins of GLP-1 analogue peptide in fusion with anti-PCSK9 antibody

[0300] Specificity for PCSK9 compared to related human proteins of the GLP-1 analogue anti-PCSK9 fusion HS9_DSB7_V19A human IgG1-TM was determined by Dissociation-Enhanced Lanthanide Fluorescent Immunoassay Time Resolved Fluorescence (DELFIA TRF Assay, PerkinElmer). Recombinant human Avi_PCSK9_Flag_His (in-house), GST tagged human PCSK7 (Abnova), GST tagged human MBTPS1 (Abnova) and Flag/His tagged human CD86 (in-house) were coated at 10 μg/mL in PBS into 96-well immunoassay plate. After washing, HS9_DSB7_V19A was added to antigen-coated wells at a concentration of 25μg/mL. Plates were incubated at room temperature for 2 hours before extensive washing. Bound HS9_DSB7_V19A human IgG1-TM was

detected using secondary Europium-labelled anti-human IgG antibody (PerkinElmer). Antigen coating to the plates was assessed by using a mouse anti-GST IgG (Abcam) as primary and Europium-labelled anti-mouse IgG (PerkinElmer) as detection for human PCSK7 and human MBTPS1. Human PCSK9 and human CD86 coating was directly assessed using a Europium-labelled anti-His IgG (PerkinElmer).

[0301] Results showed that HS9_DSB7_V19A human IgG1-TM binds strongly to human PCSK9 but not to human PCSK7, human MBTPS1 (PCSK8) or human CD86 (data not shown).

Example 19. Blocking human PCSK9 binding to LDL receptor with GLP-1 Analogue Peptide in Fusion with Anti-PCSK9 Antibody

[0302] Ability of the GLP-1 analogue anti-PCSK9 fusion HS9_DSB7_V19A to block the binding of human PCSK9 to human LDL receptor was assessed using an ELISA competition assay. Anti-PCSK9 antibody HS9 and irrelevant isotype match NIP228 human IgG1-TM were used as positive and negative controls respectively.

[0303] Binding of biotinylated human PCSK9 (in house) at 5ug/mL in 1X Phosphate Buffered Saline, 3% skimmed milk to human LDL-R (R&D Systems) coated overnight at 10ug/mL onto 96 well MaxiSorb plate (NUNC) was detected by ELISA using cryptate labelled streptavidin (Perkin Elmer) diluted at 100ng/mL in Delfia Buffer (Perkin Elmer). That interaction was challenged using a 3-fold serial dilution, starting at 100ug/mL, of compounds co-incubated for 2h at room temperature with the biotinylated PCSK9 reagent in the LDL receptor coated wells. Fluorescence signal was read on the Perkin Elmer Envision machine using a 340nm excitation and 620nm emission. Percentage of specific binding was calculated by subtracting the background signal obtained with no LDL receptor coated onto the plate normalized with the maximum specific binding signal obtained with no competitor compound minus background level.

[0304] Biochemical inhibition of PCSK9 binding to LDL receptor is presented in Figure 25.

[0305] Fusion molecule HS9_DSB7_V19A can block the binding of biotinylated human PCSK9 to recombinant LDL receptor with similar IC_{50} compared to the positive control anti-PCSK9 antibody HS9 (4.3E-8 and 3.5E-8 M respectively).

Example 20. LDL Uptake by HEPG2 Cells Treated with GLP-1 Analogue Peptide in Fusion with Anti-PCSK9 Antibody

[0306] Ability of the GLP-1 analogue anti-PCSK9 fusion HS9_DSB7_V19A to block PCSK9 activity and restore LDL uptake was tested in HepG2 hepatic cells as followed. Anti-PCSK9 antibody PC9#2 and irrelevant isotype match NIP228 human IgG1-TM were used as positive and negative controls respectively.

[0307] Human HepG2 cells were seeded in black, clear bottom 96-well Greiner plates at a concentration of $2x10^4$ cells per well in DMEM medium (Gibco) supplemented with 10% lipoprotein deficient serum (Sigma) and incubated at 37°C (5% CO_2) overnight. To complex PCSK9 with the tested compound, 45 nM of human Avi_PCSK9_FLAG_His (in house) was incubated with or without the tested compound at various concentrations in DMEM + 10% LPDS for 1 hour at room temperature. All media was removed from the cell plate and the PCSK9 / compound mixtures were transferred to the plate and incubated for 1 hour. Bodipy-LDL (Molecular Probes), diluted in DMEM + 10% LPDS to a final concentration of 50 nM, was next transferred to the cells and the plate incubated for 5 hours at 37°C (5% CO_2). Cells were washed thoroughly with PBS, stained with the nuclear dye Hoescht and fixed using formaldehyde at a final concentration of 3.7% (v/v). Assay plates were read for cell-associated fluorescence using the Cellomics ArrayScan VTi high content imaging system. Hoescht staining was measured in channel 1 using the BGRFR_386_23 filter and Bodipy-LDL in channel 2 using the BGRFR_485_20 filter. Images were analysed using the Compartmental Analysis v4 algorithm.

[0308] Inhibition of PCSK9-dependent loss of LDL uptake by HepG2 cells is presented in Figure 26.

[0309] Fusion molecule HS9_DSB7_V19A can restore LDL-uptake by HepG2 cells treated with human PCSK9 with similar IC_{50} compared to the positive control anti-PCSK9 antibody PC9#2 (2.5E-8 and 3.1E-8 M respectively).

Example 21. Potency at GLP-1 Receptors Across Species of GLP-1 Analogue Peptide in Fusion with Anti-PCSK9 Antibody

[0310] Cross-reactivity of the GLP-1 analogue anti-PCSK9 fusion HS9_DSB7_V19A at human, cynomolgus, mouse and rat GLP-1 receptors was tested in a cAMP production assay as previously described by using stable cell lines overexpressing the receptor of interest. GLP1-Fc fusion (in house) and GLP-1 peptide were used as positive controls.

[0311] Potency at the different GLP-1 receptors is summarised in Table 23.

Table 23. Potency of lead molecule HS9_DSB7_V19A at GLP-1 receptor across species

#	Compound	Potency at GLP-1R in cAMP assay across species (M)			
		human	cynomolgus	mouse	rat
1	HS9_DSB7_V19A	3.73E-09	5.83-10	5.75-10	3.83E-11
2	GLP1-Fc(Gamma4)	7.72E-11	1.09E-11	8.98E-11	8.92E-12
3	GLP-1 peptide	1.61E-11	1.03E-11	2.69E-11	9.31E-11

[0312] Fusion molecule HS9_DSB7_V19A can activate GLP-1 receptor across all the four tested species.

Example 22. Several Compounds were Identified by Reducing Potency at the Human GLP-1 Receptor

[0313] To reduce DSB7 potency, certain residues in the peptide were mutated. Peptide Ab fusions to SEQ ID NO: 2 with linker SEQ ID NO: 4 at the desired potency, here shown in green triangles, were further analyzed for specificity at human GLP1-R and species cross reactivity. See Figure 27.

[0314] The variants are shown as HS9_DSB7_G2V (Gly_2->Val) (SEQ ID NO: 44 and SEQ ID NO: 1); HS9_DSB7_E15A (Glu_{15}->Ala) (SEQ ID NO: 45 and SEQ ID NO: 1); HS9_DSB7_V19A (Val19->Ala) (SEQ ID NO: 47 and SEQ ID NO: 1); HS9_DSB7_L26I (Leu_{26}->Ile) (SEQ ID NO: 46 and SEQ ID NO:1) (compared to Val_{19}->Ala (SEQ ID NO: 3)). These four variants were selected for final characterization. Results are shown in Figure 27.

Example 23. Fusion Molecule with Reduced Potency

[0315] The PCSK9/GLP-1 fusion molecule exhibits the desired potency on the GLP-1 receptor, as shown in Figure 28A. The potency of this compound has been reduced to minimize nausea. Table 24 shows that HS9_DSB7_V19A has a 57.7 fold reduced potency with respect to dulaglutide. This engineered reduction of potency provides the desired effect of reducing nausea and other untoward effects.

Table 24: Fusion Molecule with Reduced Potency

Sample ID	Mean EC50 (M)	Fold Change Over Benchmark	Max Activation (%)
HS9_DSB7_V19A (heavy chain of SEQ ID NO: 1 and light chain fusion of SEQ ID NO: 47)	4.4E-09	57.7	98
GLP-1 (SEQ ID NO: 29)	1.6E-11	0.2	100
GLP1-Fc(G4) (dulaglutide)	7.7E-11	1.0	100

[0316] PCSK9/GLP-1 fusion of Example 1 exhibits an Ab exposure/GLP-1 activity profile sufficient to enable weekly dosing, for example, as shown in Figure 28B. pK stability study in rat of 58.5mg/kg of HS9_DSB7_V19A injected into rat. Concentration of the fusion compound is measured in the serum over time. Samples taken from rat are analyzed for both activity of the test compound and concentration of the test compound in serum. Data from the GLP-1 activity portion is used to back calculate for concentration of "active" compound. The line with closed circles is the

concentration of HS9_DSB7_V19A and line with closed squares is the concentration of active HS9_DSB7_V19A (*i.e.*, having GLP-1 activity) for the same samples.

Example 24. Specificity for GLP-1 Receptor Compared to Closely Related Human Receptors of GLP-1 Analogue Peptide in Fusion with Anti-PCSK9 Antibody

[0317] Specificity of the GLP-1 analogue anti-PCSK9 fusion HS9_DSB7_V19A for GLP-1 receptor compared to related human receptors was tested in a cAMP production assay as previously described by using stable cell lines overexpressing the receptor of interest: glucagon, GIP, GLP-2 and secretin receptors. Specific agonist peptides for each of the four receptors were used as positive controls.

[0318] Data are shown in Figure 29A-D (A: Glucagon receptor, B: GIP receptor, C: GLP-2 receptor, D: Secretin receptor).

[0319] Fusion molecule HS9_DSB7_V19A is specific for GLP-1 receptor and does not activate any of the four tested closely related receptors.

Example 25. Further Characterization of Fusion Molecule Demonstrates a Favorable *In Vivo profile.*

[0320] The PCSK9/GLP-1 fusion molecule of Example 2 (HS9_DSB7_V19A (heavy chain of SEQ ID NO: 1 and light chain fusion of SEQ ID NO: 47)) shows superior glucose control and weight loss over time, including at day 7 post dose. Data is shown against dulaglutide and PC9_2_VH and VL (SEQ ID NOS: 8 and 9). Animals were dosed at day 0 with the compounds and then their body weight was measured over time to determine a change in body weight.

[0321] The fusion molecule has shown that it binds purified PCSK9 with high affinity, it restores LDLc uptake in HEPG2 cells, stimulates GLP-1R at a desired potency, promotes weight loss and demonstrates favorable exposure/activity profile in rat PK to support weekly dosing and sustained GLP-1 activity *in vivo*.

[0322] Results are provided in Figures 30A-B.

Example 26. Impact of the Linker on the Activities of GLP-1 Analogue Peptide in Fusion With Anti-PCSK9 Antibody

[0323] The compound HS9_DSB7_V19A has a linker of SEQ ID NO: 4 corresponding to a Gly_4Ser motif repeated three times between the peptide moiety and the antibody light chain. To investigate the impact of the linker length between the GLP-1 analogue peptide DSB7_V19A (SEQ ID NO: 3) when fused to the light chain of anti-PCSK9 antibody HS9 (light chain: SEQ ID NO: 2 and heavy chain: SEQ ID NO: 1), three fusions with a reduced linker length were generated: HS9_DSB7_V19A_L2 (SEQ ID NO: 419) having a linker corresponding to the Gly_4Ser motif repeat two times (linker: SEQ ID NO: 403), HS9_DSB7_V19A_L1 (SEQ ID NO: 420) having a linker corresponding to the Gly_4Ser motif repeat one time (SEQ ID NO. 27) and HS9_DSB7_V19A_L0 with no linker between the peptide and the antibody light chain (SEQ ID NO: 421).

[0324] Compounds were tested for both binding to recombinant human PCSK9 by ELISA and activity at the human GLP-1 receptor using the cAMP assay cell based assay as described in Example 4.

[0325] Binding ELISA was performed by coating human PCSK9 (in house) at 10ug/mL in 1X Phosphate Buffered Saline. After plate blocking with 1X Phosphate Buffered Saline, 3% skimmed milk, compounds were added at 100ug/mL in PBS and incubated for 2h at room temperature before washing. Bound compounds were detected using cryptate labelled Fc specific anti human IgG (Perkin Elmer) diluted at 100ng/mL in Delfia Buffer (Perkin Elmer). Fluorescence signal was read on the Perkin Elmer Envision machine using a 340nm excitation and 620nm emission. HS9_DSB7_V19A was used as positive control and irrelevant isotype match NIP228 used to determine the background level.

[0326] PCSK9 binding and GLP-1 receptor activation data are shown in Figures 31 and 32, respectively.

[0327] All additional fusion molecules are able to bind human PCSK9 to a similar level compared to HS9_DSB7_V19A. Tested fusion molecules can also activate human GLP-1 receptor in the cAMP cell based assay but reducing linker length is having a negative impact on compound potency. In that assay, EC_{50} for HS9_DSB7_V19A, HS9_DSB7_V19A_L2, HS9_DSB7_V19A_L1 and HS9_DSB7_V19A_L0 are 5.0nM, 15.6nM, 68.7nM and 537nM, respectively.

Example 27. In Vitro Characterization of Stable GLP-1 Analogue Peptide in Fusion with Anti-PCSK9 Antibodies

[0328] The GLP-1 analogue peptide DSB7_V19A of SEQ ID NO: 3) was fused using a linker of SEQ ID NO:4 to the light chain of other anti-PCSK9 antibodies than HS9:

[0329] 1_ PC9#1 with antibody variable heavy chain of SEQ ID NO:10 and antibody variable light chain of SEQ ID NO:11

[0330] 2_ PC9#3 with antibody variable heavy chain of SEQ ID NO:404 and antibody variable light chain of SEQ ID NO:405.

[0331] 3_ PC9#4 with antibody variable heavy chain of SEQ ID NO:406 and antibody variable light chain of SEQ ID NO:407.

[0332] 4_ PC9#5 with antibody variable heavy chain of SEQ ID NO:408 and antibody variable light chain of SEQ ID NO:409.

[0333] 5_ PC9#6 with antibody variable heavy chain of SEQ ID NO:410 and antibody variable light chain of SEQ ID NO:411.

[0334] 6_ PC9#7 with antibody variable heavy chain of SEQ ID NO:412 and antibody variable light chain of SEQ ID NO:413.

[0335] Fusions were tested for both binding to recombinant human PCSK9 by ELISA as described in Example 26 and activity at the human GLP-1 receptor using the cAMP cell based assay as described in Example 4. HS9_DSB7_V19A and NIP228 isotype match were used as positive and negative controls respectively.

[0336] PCSK9 binding and GLP-1 receptor activation data are shown in Figure 33 and 34, respectively.

[0337] All seven compounds tested in the human GLP-1 receptor cAMP assay are able to activate the receptor. Potency among the panel is ranging from 1 to 350nM for PC9#6_DSB7_V19A and PC9#5_DSB7_V19A, respectively. HS9_DSB7_V19A has a potency of 5nM in that assay.

[0338] In addition, all tested fusions are also able to bind strongly to human PCSK9 by ELISA at the exception of PC9#7_DSB7_V19A which binds poorly and PC9#6_DSB7_V19A which does not bind.

Example 28. *In vitro* characterisation of stable GLP-1 analogue peptide in fusion with an anti-B7-H1 antibody

[0339] The GLP-1 analogue peptide DSB7_V19A of SEQ ID NO: 3 was fused using a linker of SEQ ID NO:4 to the light chain of the anti-B7-H1 antibody 2.7A4 described in patent WO2011066389. Anti B7-H1 antibody 2.7A4 has a variable heavy chain of SEQ ID NO:422 and a variable light chain of SEQ ID NO:423.

[0340] Fusion was tested for both binding to recombinant human B7-H1 by ELISA and activity at the human GLP-1 receptor using the cAMP assay cell based assay as described in Example 4.

[0341] Binding ELISA was performed by coating human B7-H1 (in house) at 5ug/mL in 1X Phosphate Buffered Saline. After plate blocking with 1X Phosphate Buffered Saline, 3% skimmed milk, compounds were added at 10ug/mL in PBS and incubated for 2h at room temperature before washing. Bound compounds were detected using cryptate labelled Fc specific anti human IgG (Perkin Elmer) as described in Example 25. Anti-B7-H1 antibody 2.7A4 and irrelevant isotype match NIP228 were used as positive and negative control respectively.

[0342] B7-H1 binding and GLP-1 receptor activation data are shown in Figures 35 and 36, respectively.

[0343] Fusion 2.7A4_DSB7_V19A is able to bind human B7-H1 to a similar level than the positive control 2.7A4 antibody. The fusion can also activate human GLP-1 receptor in the cAMP cell based assay with a potency of 100nM compared to 5nM for HS9_DSB7_V19A.

Example 29. Pharmacokinetics and Pharmacodynamics in Rat of GLP-1 Analogue Peptide in Fusion with Anti-PCSK9 Antibody Following Single Intravenous Dose

[0344] A PKPD study for the peptide antibody fusion HS9_DSB7_V19A following a single intravenous bolus in CD rats was conducted in order to assess its *in vivo* stability, by measuring both compound exposure and concentration in active GLP-1, as well as target engagement by measuring concentration in free rat PCSK9.

[0345] Fusion molecule was injected at 10, 30 and 60mg/kg. Anti-PCSK9 mAb HS9, without a peptide attached to it, was used as control and injected at 60mg/kg. Blood samples were collected at:

[0346] 1: Pre-dose – 0.5h – 6h – 24h – 48h – 96h and 168h for the 10mg/kg treatment group;

[0347] 2: Pre-dose – 0.5h – 24h – 48h – 96h – 168h and 240h for the 30mg/kg treatment group; and

[0348] 3: Pre-dose – 0.5h – 24h – 72h – 168h – 336h and 504h for the 60mg/kg treatment groups.

[0349] Concentrations of total human IgG1 antibody (exposure) and of active GLP-1 compound in rat serum samples were quantified as described in Example 4.

[0350] HS9_DSB7_V19A concentration over time in total and active GLP-1 compound in rat serum for the three tested doses (10, 30 and 60mg/kg) are shown in Figure 37.

[0351] Area under curve (AUC) for both total compound and active GLP-1 are summarised in Table 25. Calculating the ratio between active GLP-1 and total compound AUC is one way to evaluate *in vivo* stability. A ratio of one is corresponding to a fully stable compound in the tested conditions.

[0352] Data for the parent fusion molecule PC9#2_Exe4 comprising Exendin-4 in light chain fusion with the anti-PCSK9 mAb PC9#2 (See Example 4) have also been included for comparison.

Table 25. Total and active GLP-1 AUC0-t following a single IV injection of the fusion molecule HS9_DSB7_V19A compared to the parent fusion molecule PC9#2_Exe4

Compound	Design	AUC0-t Total (day·nmol)	AUC0-t Active GLP-1 (day·nmol)	Active/ Total AUC Ratio
HS9_DSB7_V19A	10 mg/kg IV	1595	1216	0.76
HS9_DSB7_V19A	30 mg/kg IV	8702	6230	0.72
HS9_DSB7_V19A	60 mg/kg IV	24535	26494	1.08
PC9#2_Exe4	1 mg/kg IV	299	40	0.13

[0353] HS9_DSB7_V19A displays a greater Active/Total AUC ratio compared to the parent molecule PC9#2_Exe4 demonstrating an improved *in vivo* stability profile in rat for activity at GLP-1 receptor.

[0354] Determination of free rat PCSK9 concentration in serum samples was based on a sandwich ligand binding assay method using the MSD® platform. Free rat PCSK9 was captured using the anti-PCSK9 mAb HS9 that was first non-specifically adsorbed on to the carbon surface of a Standard Bind MSD® plate. Anti-PCSK9 antibody from Abcam (Product Number ab125251) was labelled in-house with MSD® SULFO-TAG™ and used as detection reagent. Plate was then read using the MSD® Sector Imager 6000 (SI6000) instrument.

[0355] Engagement of HS9_DSB7_V19A to rat PCSK9 over time was evaluated by measuring the concentration of free antigen in the rat serum samples. Data for the three dosing groups (10, 30 and 60 mg/kg) are shown in Table 26 and Figure 38.

Table 26: Concentration of Free Antigen in Rat Plasma Samples Over Time

10 mg/kg IV		30 mg/kg IV		60 mg/kg IV	
Time (h)	**Mean free rat [PCSK9] in ng/mL**	**Time (h)**	**Mean free rat [PCSK9] in ng/mL**	**Time (h)**	**Mean free rat [PCSK9] in ng/mL**
0	837.2	0	741.8	0	688.5
0.5	below LLOQ	0.5	125.8	0.5	below LLOQ
6	26.7	24	below LLOQ	24	below LLOQ
24	74.1	48	below LLOQ	72	below LLOQ
48	162.5	96	below LLOQ	168	below LLOQ
96	301.0	168	70.9	336	45.4
168	374.7	240	130.3	504	135.8

[0356] HS9_DSB7_V19A is able to suppress rat PCSK9 below 90% at all tested doses but the duration of suppression is dose dependent. Free rat PCSK9 is again detectable at 6h, 168h and 336h after injection when HS9_DSB7_V19A is dosed at 10, 30 and 60mg/kg respectively.

Example 30. Pharmacokinetics and Pharmacodynamics in Rat of GLP-1 Analogue Peptide in Fusion with Anti-PCSK9 Antibody Following Single Subcutaneous Dose

[0357] A pharmacokinetic study for the GLP-1 analogue peptide antibody fusion HS9_DSB7_V19A following a single subcutaneous bolus at 60mg/kg in CD rats was performed in order to compare routes of administration and their potential impact on compound exposure and *in vivo* stability.

[0358] Fusion molecule was injected at 40mg/mL using a 1.5mL/kg regimen. Blood samples were collected at Pre-dose – 6h – 24h – 48h – 96h – 168h and 240h.

[0359] Concentrations of total human IgG1 antibody and of active GLP-1 compound as well as concentrations in free rat PCSK9 antigen in the serum samples were determined as described in Example 4 and Example 29.

[0360] Total and active compound for HS9_DSB7_V19A and free rat PCSK9 concentrations are shown in Figure 39. A maximum compound concentration of around 1000nM was observed between 48h and 96h post-injection. Concentration in free rat PCSK9 is dropping sharply below the lower limit of quantification of the assay after compound injection and can be detected again at 168 h.

[0361] Area under curve (AUC) for both total and active compound were calculated and compared (Table 27) to model prediction using the data from the single intravenous dose injection described in Example 29. Fraction of absorption and absorption rate were set up to 75% and 0.3 d^{-1} respectively.

Table 27: Calculated and predicted Area Under Curve following a single SC injection of the fusion molecule HS9_DSB7_V19A		
	SC data 60mg/kg	**Predicted using IV data**
Exposure AUC0-t (day nmol)	7815	8103
Active GLP-1 AUC0-t (day nmol)	5796	5739
Active/Exposure AUC ratio	0.74	0.71

[0362] Exposure and active GLP-1 AUC of HS9_DSB7_V19A after a single subcutaneous injection at 60mg/kg are similar to those predicted using the single intravenous injection data. This demonstrates that a subcutaneous route of injection has no significant impact on *in vivo* compound stability compared to an intravenous route of administration.

Example 31. Rodent Pharmacology-Antidiabetic Effects of a GLP-1 Analogue Peptide in Fusion with an Anti-PCSK9 Antibody

[0363] In order to confirm that the engineered nature of the GLP-1 analogue peptide portion of the HS9_DSB7_V19A fusion molecule retained antidiabetic activity in vivo, several; rodent pharmacology studies in normal, obese, and diabetic mouse models were performed.

A) Acute and semi-acute effects of HS9_DSB7_V19A on glucose tolerance in C57Bl6 mice

[0364] A single dose of HS9_DSB7_V19A was administered subcutaneously in normal C57Bl6 mice at either 1 or 50 mg/kg. The efficacy of the GLP-1 analogue component of the fusion molecule was evaluated by multiple glucose challenges (oral glucose tolerance test) at 4, 48, and 168 hours post administration of HS9_DSB7_V19A. Anti-PCSK9 mAb HS9 without a GLP-1 analogue peptide fused to it was administered at 50 mg/kg as a negative control for glucose tolerance, while Liraglutide (Victoza) and a GLP-1 analogue-Fcγ4 fusion (similar to Dulaglutide) were administered at 0.2 and 1 mg/kg, respectively. Due to the short half-life of Liraglutide, this compound was

administered 2 hours prior to each glucose challenge while the GLP-1 analogue-Fcγ4 fusion molecule was administered once, at the same time as the HS9_DSB7_V19A test compound.

[0365] 30 male C57Bl6 mice from Taconic Denmark were acclimatized for five days before experimentation. On day -1 of the study, animals were randomized into 5 groups based on body weight.

[0366] The experimental groups were as follows:

[0367] Group 1: Anti-PCSK9 mAb HS9 without GLP-1 analogue peptide component (negative control) - 50 mg/kg subcutaneous dose

[0368] Group 2: Liraglutide (positive control) – 0.2 mg/kg subcutaneous dose

[0369] Group 3: HS9_DSB7_V19A – 1 mg/kg subcutaneous dose

[0370] Group 4: HS9_DSB7_V19A – 50 mg/kg subcutaneous dose

[0371] Group 5: GLP-1 analogue-Fcγ4 fusion - 1 mg/kg subcutaneous dose

[0372] In order to assess efficacy of the GLP-1 analogue component of the fusion molecule over an extended period of time post dosing, 3 Oral Glucose Tolerance Tests (OGTT) were performed at days 0, 2 and 7. Animals were fasted for 4 hours prior to the oral glucose challenge(s). Both doses of HS9_DSB7_V19A, inactive control and GLP-1 analogue-Fcγ4 fusion were administered once, 4 hours prior to the Day 0 OGTT. Liraglutide (positive control) was administered 2 hours prior to each glucose challenge at days 0, day 2 and day 7. At t = 0 mice all mice are challenged with an oral glucose load of 2g/kg glucose. Blood glucose is measured at t = -240, -120 and 0 minutes to establish a baseline and at t = 15, 30, 60 and 120 minutes to monitor effects on glucose excursion. The results of the day 0, 2 and 7 OGTTs are presented in Figures 40A-C (day 0 (A), day 2 (B), day 7 (C)).

[0373] This study confirms the ability of a single, subcutaneous administration of HS9_DSB7_V19A at 50 mg/kg to improve glucose tolerance in normal C57Bl6 mice for at least 7 days.

[0374] As an additional measure of efficacy of the GLP-1 analogue component of the HS9_DSB7_V19A fusion molecule body weights of all were recorded once daily from day -3 to the end of the study. A single, subcutaneous administration of HS9_DSB7_V19A at 50 mg/kg induced a transient reduction in body weight at days 1 and 2. Percent body-weight change over time is shown in Figure 41.

B) Acute and semi-acute effects of HS9_DSB7_V19A on glucose tolerance in C57Bl6 mice-dose response

[0375] In order to examine a dose response effect and to determine a maximally efficacious dose of HS9_DSB7_V19A on glucose tolerance and body weight reduction in normal C57Bl6 mice, a study similar in design to that described above was conducted with the following experimental groups and doses:

[0376] Group 1: Anti-PCSK9 mAb HS9 without GLP-1 analogue peptide component (negative control) - 50 mg/kg subcutaneous dose

[0377] Group 2: Liraglutide (positive control) – 0.2 mg/kg subcutaneous dose

[0378] Group 3: HS9_DSB7_V19A – 10 mg/kg subcutaneous dose

[0379] Group 4: HS9_DSB7_V19A – 30 mg/kg subcutaneous dose

[0380] Group 5: HS9_DSB7_V19A – 60 mg/kg subcutaneous dose

[0381] Group 6: GLP-1 analogue-Fcγ4 fusion - 1 mg/kg subcutaneous dose

[0382] On day -1 animals were randomized into these 6 groups based on body weight (n = 6 animals per group). As previously, OGTTs were performed on Days 0, 2 and 7 and blood glucose was measured at t = -240, -120, 0, 15, 30, 60 and 120 minutes.

[0383] All three doses of HS9_DSB7_V19A resulted in similar levels of improved glucose tolerance at all three time points at which OGTTs were performed. Figures 42A-C illustrate the results from this oral glucose tolerance tests (day 0 (A), day 2 (B), day 7 (C)).

[0384] In contrast to the lack of a dose response observed for improvements in glucose homeostasis, a clear dose-dependent effect on body weight reduction was observed in this study. Figure 43.

[0385] In this experimental model a single 10 mg/kg dose of HS9_DSB7_V19A generated a maximally efficacious level of improvement in glucose homeostasis in an OGTT performed 7 days post administration while the same dose did not have a statistically significant effect on body weight reduction at any point during the study.

C) Chronic metabolic effects of HS9_DSB7_V19A in a diabetic *db/db* mouse model

[0386] In order to confirm the chronic vivo efficacy of HS9_DSB7_V19A on several metabolic parameters in a diabetic model, *db/db* (leptin receptor deficient) mice were utilized to examine the effects of weekly administration of HS9_DSB7_V19A on fasting glucose, glucose tolerance, body weight reduction and body mass composition.

[0387] In this study we examined the chronic metabolic effects of HS9_DSB7_V19A upon weekly dosing via a subcutaneous route. The vehicle control group and the positive control GLP-1 analogue-Fcγ4 fusion molecule were subcutaneously dosed twice weekly. In order to match the number of dosing manipulations to all animals in the study, the groups receiveing a weekly dose of HS9_DSB7_V19A were dosed with vehicle on the days the other animals received their second weekly dose of either vehicle (negative control) or positive control GLP-1 analogue-Fcγ4 fusion molecule.

[0388] 60 male *db/db* mice from Charles River, Italy were primarily randomized on body weight and glycosylated hemoglobin (HbA1c) and secondarily on 4 hour fasting blood glucose. These animals were randomized into 5 groups (n=12) as follows:

[0389] Group 1: Vehicle control group (Phosphate Buffered Saline) – twice weekly (BIW) subcutaneous dosing

[0390] Group 2: GLP-1 analogue-Fcγ4 fusion (positive control) - 1 mg/kg subcutaneous dose – twice weekly subcutaneous dosing

[0391] Group 3: HS9_DSB7_V19A – 30 mg/kg subcutaneous dose – once weekly (QW)

[0392] Group 4: HS9_DSB7_V19A – 10 mg/kg subcutaneous dose – once weekly (QW)

[0393] Group 5: HS9_DSB7_V19A – 3 mg/kg subcutaneous dose – once weekly (QW)

[0394] The chronic study was run for 28 days post initial dose. Last dose of the BIW groups was on study day 24 whereas the last dose of the QW groups was on study day 21. The major efficacy endpoints of this study are body weight, fasting blood glucose, and glucose tolerance.

[0395] Body weight was measured three times weekly during the treatment period.

[0396] 4 hour fasting blood glucose was measured once weekly at approximately 24 hours post dosing.

[0397] Glucose tolerance was measured on study day 22 at approximately 24 hours post dosing by Intraperitoneal glucose tolerance test (IPGTT). Animals were fasted for 4 hours before administration of a 1g/kg glucose bolus. Blood glucose was measured at t = 0, 15 , 30, 60, 120, and 180 minutes.

[0398] Body weight reduction in this study was not significant with the exception of one time point in the positive control GLP-1 analogue-Fcγ4 fusion molecule group. All three doses of HS9_DSB7_V19A did not result in a significant body weight reduction at any time during the course of the 28 day study. Figure 44.

[0399] Weekly HS9_DSB7_V19A exhibited a dose dependent reduction in 4 hour fasting blood glucose as compared to vehicle control. Figure 45.

[0400] Weekly dosed HS9_DSB7_V19A exhibited a dose dependent improvement in glucose tolerance as assessed by IPGTT at study day 22. Figure 46.

D) Acute effect of single dose HS9_DSB7_V19A on glucose tolerance in a Diet-Induced Obesity (DIO) mouse model

[0401] A single dose of HS9_DSB7_V19A was administered subcutaneously in Diet-induced obese (DIO) mice at 0.1, 1 or 10 mg/ kg. The efficacy of the GLP-1 analogue component of the fusion molecule was evaluated by separate glucose challenges (intraperitoneal glucose tolerance test) at 4 and 168 hours post administration of HS9_DSB7_V19A. Anti-PCSK9 mAb HS9 without a GLP-1 analogue peptide fused to it was administered once at 10 mg/kg as a negative control for effects on glucose tolerance. As a positive control, the GLP-1 analogue-Fcγ4 fusion (similar to Dulaglutide) used in the previous experiments was administered at 1 mg/kg twice weekly (BIW). In order to simulate the dosing regimen of the positive control GLP-1 analogue-Fcγ4 fusion, all animals in the negative control group and the HS9_DSB7_V19A experimental groups were dosed with vehicle BIW. The study duration was for 21 days following the first dose. Primary endpoint was effects on glucose tolerance in IPGTT on day 0 (4 hours post dose) and Day 7. Secondary endpoint was body weight reduction.

[0402] 50 Male, 21 week old DIO mice on 60% high fat diet for 15 weeks prior to study start were obtained from Jackson labs (JAX: 380050). Just prior to study start, animals were randomized into 5 groups based on body weight.

[0403] The experimental groups (n=10 per group) were as follows:

[0404] Group 1: Anti-PCSK9 mAb HS9 without GLP-1 analogue peptide component (negative control) - 10 mg/kg subcutaneous dose – single dose

[0405] Group 2: HS9_DSB7_V19A – 0.1 mg/kg subcutaneous dose – single dose

[0406] Group 3: HS9_DSB7_V19A – 1 mg/kg subcutaneous dose –single dose

[0407] Group 4: HS9_DSB7_V19A – 10 mg/kg subcutaneous dose – single dose

[0408] Group 5: GLP-1 analogue-Fcγ4 fusion - 1 mg/kg subcutaneous dose – twice weekly dosing (BIW)

[0409] In order to assess efficacy of the GLP-1 analogue component of the fusion molecule over an extended period of time post dosing, two Intraperitoneal Glucose Tolerance Tests (IPGTT) were performed at days 0 and 7. Animals were fasted for 6 hours prior to the IP glucose challenge(s). All three doses of HS9_DSB7_V19A, inactive control and GLP-1 analogue-Fcγ4 fusion were administered once, 4 hours prior to the Day 0 OGTT. The GLP-1 analogue-Fcγ4 fusion was administered on days 0, 3, 7, 10, 14 17 and 21. On day 0 all groups were dosed with test compounds 4 hours prior to the IP glucose challenge while on day 7 the GLP-1 analogue-Fcγ4 fusion was administered 4 hours prior to the IP glucose challenge while all other groups were dosed with vehicle only . For each day in which IPGTTs were performed, at t = 0 mice all mice were challenged with an IP glucose load of 1.5g/kg glucose. Blood glucose was measured at t = -240 and 0 minutes to establish a baseline and at t = 15, 30, 45, 60, 90 and 120 minutes to monitor effects on glucose excursion.

[0410] The results of the day 0 and 7 IPGTTs are presented in Figure 47A-B.

[0411] In order to assess effects of the GLP-1 component of HS9_DSB7_V19A on body weight, all animals were weighed daily for the entire course of the study. Effects on body weight are presented in 48A-B.

E) Effects of multiple doses of HS9_DSB7_V19A on body weight in DIO mice.

[0412] In order to establish a dose-response in body weight reduction HS9_DSB7_V19A was administered subcutaneously to diet-induced obese mice at 3, 10 and 30 mg/kg once weekly (QW). The duration of the study was 28 days and primary endpoint was body weight reduction. Secondary evaluation of glycemic parameters included analyses included fed glucose throughout the course of the study and measurements of terminal fasting glucose. As in our previous studies in both DIO and db/db mice, the GLP-1 analogue-Fcγ4 fusion was administered subcutaneously, twice weekly (BIW) at 1 mg/kg as a positive control for both glucose control and body weight loss.

[0413] Male, 21 week old DIO mice on 60% high fat diet for 15 weeks prior to study start were obtained from Jackson labs (JAX: 380050). Just prior to study start, animals were randomized into 5 groups based on body weight (n = 8 animals per group).

[0414] The experimental groups (n = 8 per group) were as follows:

[0415] Group 1: Vehicle control

[0416] Group 2: HS9_DSB7_V19A – 3 mg/kg subcutaneous dose – once weekly (QW)

[0417] Group 3: HS9_DSB7_V19A – 10 mg/kg subcutaneous dose – once weekly (QW)

[0418] Group 4: HS9_DSB7_V19A – 30 mg/kg subcutaneous dose – once weekly (QW)

[0419] Group 5: GLP-1 analogue-Fcγ4 fusion - 1 mg/kg subcutaneous dose – twice weekly dosing (BIW)

[0420] In order to assess effects of the GLP-1 component of HS9_DSB7_V19A on body weight (the primary endpoint of this study), all animals were weighed daily for the entire course of the study. Effects on body weight are presented in Figure 49.

[0421] As a secondary endpoint and in order to assess effects of the GLP-1 component of HS9_DSB7_V19A on glycemic control in a weekly dose setting, fed glucose was measured at days 0,

7, 11, 14, 21 and 26 and fasting glucose was measured at just prior to study termination (day 28). Results are presented in Figure 50A-B.

EQUIVALENTS

[0422] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the embodiments. The foregoing description and Examples detail certain embodiments and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the embodiments may be practiced in many ways and the claims include any equivalents thereof.

WHAT IS CLAIMED IS:

1. A dual active fusion molecule for the treatment of diabetes comprising an anti-PCSK9 antibody stably fused to a GLP-1 peptide, wherein the anti-PCSK9 antibody binds a PCSK9 polypeptide and the GLP-1 peptide binds a GLP-1 receptor.

2. The fusion molecule of claim 1 wherein the GLP-1 peptide is fused to the PCSK9 antibody via a linker peptide.

3. The fusion molecule of claim 2 wherein the linker peptide is fused to the C-terminus of the GLP-1 peptide.

4. The fusion molecule of any one of claims 1-3, wherein the GLP-1 peptide comprises the amino acid sequence of SEQ ID NO: 36.

5. The fusion molecule of any one of claims 1-3, wherein the GLP-1 peptide comprises the amino acid sequence of SEQ ID NO: 3.

6. The fusion molecule of claim 5 wherein the Cys18 of the GLP-1 peptide forms a disulfide bridge with the C terminus of the GLP-1 peptide.

7. The fusion molecule of any one of claims 1-6, wherein said fusion molecule controls glucose and/or reduces LDL in an animal.

8. The fusion molecule of claim 7, wherein the animal is a human.

9. A dual active fusion molecule for the treatment of diabetes comprising an anti-PCSK9 antibody stably fused to a GLP-1 peptide comprising the amino acid sequence of SEQ ID NO: 3, wherein the C-terminus of the GLP-1 peptide is fused via a peptide linker to the anti-PCSK9 antibody, and wherein the anti-PCSK9 antibody binds a PCSK9 polypeptide and the GLP-1 peptide binds a GLP-1 receptor.

10. The dual active fusion molecule of claim 9, wherein the GLP-1 peptide is fused via a peptide linker to the light chain of the anti-PCSK9 antibody.

11. The dual active fusion molecule of claim 9, wherein the GLP-1 peptide is fused via a peptide linker to the heavy chain of the anti-PCSK9 antibody.

12. A dual active fusion molecule comprising an antibody stably fused to a GLP-1 peptide comprising the amino acid sequence of SEQ ID NO: 3, wherein the C-terminus of the GLP-1 peptide is fused via a peptide linker to the antibody, and wherein the antibody binds a target polypeptide and the GLP-1 peptide binds a GLP-1 receptor.

13. The dual active fusion molecule of claim 12, wherein the antibody is an anti-PCSK9 antibody.

14. The dual active fusion molecule of claim 13, wherein the GLP-1 peptide is fused via a peptide linker to the light chain of the anti-PCSK9 antibody.

15. The dual active fusion molecule of claim 13, wherein the GLP-1 peptide is fused via a peptide linker to the heavy chain of the anti-PCSK9 antibody.

16. The dual active fusion molecule of claims 10 or 14, wherein the light chain of the anti-PCSK9 antibody is at least 90% identical to the amino acid sequence of SEQ ID NO: 2.

17. The dual active fusion molecule of claim 16, wherein the light chain of the anti-PCSK9 antibody comprises the amino acid sequence of SEQ ID NO: 2.

18. The dual active fusion molecule of claims 11 or 15, wherein the heavy chain of the anti-PCSK9 antibody is at least 90% identical to the amino acid sequence of SEQ ID NO: 1.

19. The dual active fusion molecule of claim 18, wherein the heavy chain of the anti-PCSK9 antibody comprises the amino acid sequence of SEQ ID NO: 1.

20. The dual active fusion molecule of any one of claims 12-19 wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 4.

21. The dual active fusion molecule of any one of claims 12-20, wherein the dual active fusion molecule is for the treatment of diabetes.

22. The dual active fusion molecule of any one of claims 12-21, wherein the Cys18 of the GLP-1 peptide forms a disulfide bridge with the C terminus of the GLP-1 peptide.

23. The dual active fusion molecule of any one of claims 12-22, wherein the dual active fusion molecule controls glucose and/or reduces LDL in an animal.

24. The dual active fusion molecule of claim 23, wherein the animal is a human.

25. A dual active fusion molecule for the treatment of diabetes comprising an anti-PCSK9 antibody stably fused to a GLP-1 peptide that has reduced potency at the human GLP-1 receptor compared to a GLP-1 peptide comprising the amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 29, wherein the C-terminus of the GLP-1 peptide is fused via a peptide linker to the anti-PCSK9 antibody, and wherein the anti-PCSK9 antibody binds a PCSK9 polypeptide and the GLP-1 peptide binds a GLP-1 receptor.

26. The dual active fusion molecule of claim 25, wherein the GLP-1 peptide comprising the amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 29 is fused using a linker comprising SEQ ID NO:4 to the amino acid sequence comprising SEQ ID NO: 416.

27. The dual active fusion molecule of claims 25 or 26, wherein the potency at the human GLP-1 receptor is reduced by 30 to 60 fold compared to a GLP-1 peptide comprising the amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 29.

28. A method of treating Type 2 Diabetes comprising administering to a subject in need thereof, a fusion molecule of any one of claims 1-27.

29. A method of controlling glucose in a subject comprising administering to a subject in need thereof, a fusion molecule of any one of claims 1-27.

30. A method of reducing low density lipoprotein (LDL) in a subject comprising administering to a subject in need thereof, a fusion molecule of any one of claims 1-27.

31. A method of controlling glucose and reducing LDL in a subject comprising administering to a subject in need thereof, a fusion molecule of any one of claims 1-27.

32. A method of promoting weight loss, controlling glucose and reducing LDL in a subject comprising administering to a subject in need thereof, a fusion molecule of any one of claims 1-27.

33. The method of any one of claims 28-32, wherein the subject has Type 2 diabetes.

34. The method of any one of claims 28-32, wherein the subject has metabolic syndrome.

35. An isolated polynucleotide encoding the fusion molecule of any one of claims to 27.

36. A vector comprising the polynucleotide of claim 35.

37. A host cell comprising the polynucleotide of claim 35 or the vector of claim 36.

38. A method of making the fusion molecule of any one of claims to 27, comprising culturing the host cell of claim 35 under conditions allowing expression of the fusion molecule, and recovering the fusion molecule.

39. A pharmaceutical composition comprising the fusion molecule of any one of claims 1 to 27, and a carrier.

40. A kit comprising the composition of claim 39.