Antibody conjugates and fusion proteins

Field of the invention

The present invention relates to immunoglobulins, such as immunoconjugates, comprising an ActRII receptor antibody and an agonistic GLP-1 molecule, such as agonistic GLP-1 peptides. Such constructs are useful in the treatment of numerous diseases and disorders, including metabolic disorders, such as obesity, diabetes and others.

Background of the invention

Activin type 2 receptors belong to a larger TGF-beta receptor family and modulate signals for transforming growth factor beta ligands. These receptors are involved in a host of physiological processes including, growth, cell differentiation, homeostasis, osteogenesis, apoptosis and many other functions. There are two activin type two receptors: ActRIIA (ACVR2A) and ActRIIB (ACVR2B).

Despite the large amount of processes that these ligands regulate, they all operate through essentially the same pathway: A ligand binds to a type 2 receptor, which recruits and trans-phosphorylates a type I receptor. The type I receptor recruits a receptor regulated SMAD (R-SMAD) which it phosphorylates. The R-SMAD then translocates to the nucleus where it functions as a transcription factor.

It was reported that several ligands that signal through the activin type 2 receptors regulate muscle growth (Proc. Natl. Acad. Sci. U.S.A. 102 (50): 18117–22).

Glucagon-like peptide-1 (GLP-1) is a peptide hormone deriving from the tissue-specific posttranslational processing of the proglucagon peptide. It is produced and secreted by intestinal enteroendocrine L-cells and certain neurons within the nucleus of the solitary tract in the brainstem upon food consumption. The initial product GLP-1 (1–37) is susceptible to amidation and proteolytic cleavage, which gives rise to the two truncated and equipotent biologically active forms, GLP-1 (7–36) amide and GLP-1 (7–37). Active GLP-1 protein secondary structure includes two α-helices from amino acid position 13–20 and 24–35 separated by a linker region.

Alongside glucose-dependent insulinotropic peptide (GIP), GLP-1 is an incretin; thus, it has the ability to decrease blood sugar levels in a glucose-dependent manner by enhancing the secretion of

insulin. Besides the insulinotropic effects, GLP-1 has been associated with numerous regulatory and protective effects. Unlike GIP, the action of GLP-1 is preserved in patients with type 2 diabetes. Glucagon-like peptide-1 receptor agonists gained approval as drugs to treat diabetes and obesity starting in the 2000s.

Combination treatments comprising ActRII receptor antibodies and a glucagon-like peptide- 1 receptor (GLP-1) agonist were reported (WO2023/028606), but the individual moieties (i.e. the ActRII receptor antibody and the glucagon-like peptide- 1 receptor (GLP-1) agonist) are separated, unconjugated molecules. The present invention provides conjugates comprising ActRII receptor antibodies and glucagon-like peptide- 1 receptor (GLP-1) agonists, i.e. as a single molecule. Therefore only one individual molecule must be developed, as compared to two in a combination therapy. Drug formulations are finely tuned for individual compounds and cannot be applied generally. In addition, individual molecules do have very distinct ADME profiles which need to be individually considered for combination therapies. Furthermore, pharmacological synergy can often only be obtained when molecules act at the same time, in the same tissue with the same concentration. This is only achievable when polypharmacology is designed into a single entity. Furthermore, the immunoconjugate described herein allows the targeting of GLP-1 peptides to tissues where the ActR2 receptors are expressed, such as muscle and heart. At the same time, the ligation of GLP-1 agonists to the ActRII receptor antibody substantially improves the ADME of the peptide and e.g. increases the duration of action. Additionally it was surprisingly found that the immunoconjugates, unlike semaglutide, led to a loss of body weight while fully maintaining lean body mass.

Figure legends

Figure 1 shows a capillary electrophoresis gel view of end products of antibodies “E272C” (lanes 1 and 4) and “wildtype” (lanes 2 and 5) under nonreducing (lanes 1 and 2) and reducing (lanes 4 and 5) conditions. Size marker is shown in lane 3. CE-SDS was conducted on LabChip GXII Touch HT Protein Characterization System (PerkinElmer) using LabChip® GXII Touch™ HT Chip (PerkinElmer) and ProteinEXact Assay Reagent Kit (PerkinElmer) according to manufacture’s instructions. 2.5 µg of end product was taken into analysis and samples were heated at 70 °C for 10 minutes before injection.

Figure 2 shows that conjugation of peptides to the antibodies does not affect binding of the antibody to ActRIIA (panel A) and ActRIIB (panel B).

Figure 3 shows that conjugation of peptides to the antibodies does not affect activity when measured either against Activin A (panel A) or Myostatin (panel B).

Figure 4 shows that conjugation of peptides to the antibodies does not affect activity of the conjugates in a GLP- 1 cAMP assay.

Figure 5: DIO mice were treated with vehicle, semaglutide, E272C mAb-peptide 5 or E272C mAb-peptide 6 for 17 days. Bodyweight (panel A), food intake (panel B), fat mass (panel C) and lean mass (panel D) of the animals were measured.

Figure 6 shows the fusion constructs tested in Example 6.

Summary of the invention

The present disclosure relates to an immunoconjugate comprising an ActRII receptor antibody and an agonistic GLP-1 peptide.

The ActRII receptor antibody comprised in said immunoconjugate may be specific for a polypeptide of SEQ ID No. 12. Said ActRII receptor antibody may be cross-reactive with a polypeptide of SEQ ID No. 11. Said ActRII receptor antibody may comprise the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3). Said ActRII receptor antibody may bind to the to the same epitope as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3). Said ActRII receptor antibody may have a silencing mutation. Said ActRII receptor antibody may have a LALA silencing mutation. Said ActRII receptor antibody may have a PA-LALA silencing mutation.

The agonistic GLP-1 peptide comprised in said immunoconjugate may be selected from

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-K(MiniPEGAcBr) (SEQ ID No. 14),

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-KKgGluC16(MiniPEGAcBr) (SEQ ID No. 15),

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-K(GGGGS)3AcBr (SEQ ID No. 16),

HAibEGTFTSDVSSYLEGQAA-K(MiniPEGAcBr)-EFIAWLVRGRG (SEQ ID No. 17),

HAibEGTFTSDVSSYLEGQAA-KKgGluC16(MiniPEGAcBr)-EFIAWLVRGRG (SEQ ID No. 18),

HAibEGTFTSDVSSYLEGQAA-K(GGGGS)3AcBr-EFIAWLVRGRG (SEQ ID No. 19),

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-K(MiniPEGAcBr) (SEQ ID No. 20),

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-KKgGluC16(MiniPEGAcBr) (SEQ ID No. 21), and

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-K(GGGGS)3AcBr (SEQ ID No. 22).

Said agonistic GLP-1 peptide may also be a GLP-1 dual agonist or a GLP-1 triagonist. Said GLP-1 peptide may also be selected from

YAibEGTFTSDYSIYLDKQAAAibEFVNWLLAGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 23),

YAibEGTFTSDYSIYLDKQAAAibEFV-K(MiniPEGAcBr)-WLLAGGPSSGAPPPSK-NH2 (SEQ ID No. 24),

YAibEGTFTSDYSIYLDKQAAAibEFVNWLLAG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 25),

YAibEGTFTSDYSIYLDKQAAAibEFV-K(MiniPEGAcBr)-WLLAG-NH2 (SEQ ID No. 26),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 27),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 28),

YAibAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 29),

YAibQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 30),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSarGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 31),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSar-K(MiniPEGAcBr)-NH2 (SEQ ID No. 32),

YAibAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSarGPSSGAPPPSK(MiniPEGAcBr)-NH2 (SEQ ID No. 33),

YAibQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSar-K(MiniPEGAcBr)-NH2 (SEQ ID No. 34),

Ac-hAQGTFTSD-K(MiniPEGAcBr)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-NH2 (SEQ ID No. 35),

Ac-hAQGTFTSDKSKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 36),

Ac-hAQGTFTSD-K(MiniPEGAcBr)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(gGluC16)-NH2 (SEQ ID No. 37), and

Ac-hAQGTFTSD-K(gGluC16)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 38).

Said agonistic GLP-1 peptide may be conjugated to said ActRII receptor antibody via cysteine engineering. Said agonistic GLP-1 peptide may be conjugated to said ActRII receptor antibody via cysteine engineering at position 272, 361, 384, 389 or 434 of the ActRII receptor antibody. Said agonistic GLP-1 peptide may also be conjugated to said ActRII receptor antibody via a cysteine residue located in the hinge region.

The present disclosure also relates to a pharmaceutical composition comprising aforementioned immunoconjugates.

The present disclosure also relates to aforementioned immunoconjugates or aforementioned pharmaceutical composition for use in medicine. Said use in medicine may be the use in the treatment of a metabolic disorder. Said metabolic disorder may be selected from the group consisting of: obesity, diabetes, metabolic syndrome, anti-psychotic drug-associated obesity, glucocorticoid-induced obesity, hypothalamic obesity associated with craniopharyngioma, Prader-Willi syndrome, and a monogenetic disorder associated with obesity. Said use in medicine may also be a treatment useful for an obesity related co-morbidity, wherein the condition is selected from the group of: glucose intolerance, prediabetes, insulin resistance, high triglycerides, overweight associated physical impairment, osteoporosis, renal disease, obstructive sleep apnea, sexual hormones impairment, endocrine reproductive disorders, osteoarthritis, gastrointestinal cancers, dyslipidemia, hypertension, heart failure, coronary heart disease, stroke, and/or gallstones.

The present disclosure also relates to an ActRII receptor antibody, wherein said ActRII receptor antibody is fused to an agonistic GLP-1 peptide.

Embodiments of the invention

Antibody

The term "**antibody**" as used herein refers to a protein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, which interacts with an antigen. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain

constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FR's arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. The term "antibody" includes for example, monoclonal antibodies, human antibodies, humanized antibodies, camelised antibodies and chimeric antibodies. The antibodies can be of any isotype (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgD , IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass. Both the light and heavy chains are divided into regions of structural and functional homology.

The terms "**binds specifically to**", "**specifically binds to**", is "**specific to/for**" or "**specifically recognizes**" in the context of an antibody, refers to an antibody that is specific for an antigen and is able to discriminate between the target antigen antigen and one or more reference antigen(s). Specificity is not an absolute, but a relative property. For example, a standard ELISA assay or standard flow cytometry assay can be carried out. The scoring may be carried out by standard color development (e.g. secondary antibody with horseradish peroxide and tetramethyl benzidine with hydrogen peroxide) or by binding of a secondary antibody labeled with PE or another dye or marker. The reaction in certain wells is scored by the optical density (OD), for example, at 450 nm or by mean fluorescence intensity (MFI) in flow cytometry. Typical background (=negative reaction) may be 0.1 OD; typical positive reaction may be 1 OD. Background and positive reaction MFI are highly dependent on instrument settings. The difference positive/negative can be more than 10-fold. Typically, determination of binding specificity is performed by using not a single reference antigen, but a set of about three to five unrelated antigens, such as milk powder, BSA, transferrin or the like. For flow cytometry various antigen-negative cells can be used. An antibody that specifically binds to an antigen may however have cross-reactivity to the respective orthologous antigen from other species (e.g., species homologs). In certain embodiments such cross-reactivity to an orthologous antigen is even preferred.

As used herein, an antibody has "**cross-reactivity**" or is "**cross-reactive**" if it binds to a closely related antigen or the same antigen from other species. In the present disclosure the term is used for an antibody that is specific for ActRIIB but that also binds to ActRIIA.

The term "**ActRII receptor antibody**" as used herein refers to an antibody specific for ActRIIA, antibodies specific for ActRIIB, and antibodies specific for ActRIIA and ActRIIB, i.e. antibodies with a cross-reactivity between ActRIIA and ActRIIB.

The terms "**ActRIIA**" and "**ACVR2A**" as used herein refers to the human protein with UniProt ID P27037. ActRIIA has the following amino acid sequence:

MGAAAKLAFAVFLISCSSGAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHC
FATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEM
EVTQPTSNPVTPKPPYYNILLYSLVPLMLIAGIVICAFWVYRHHKMAYPPVLVPTQDPGP
PPPSPLLGLKPLQLLEVKARGRFGCVWKAQLLNEYVAVKIFPIQDKQSWQNEYEVYSLPG
MKHENILQFIGAEKRGTSVDVDLWLITAFHEKGSLSDFLKANVVSWNELCHIAETMARGL
AYLHEDIPGLKDGHKPAISHRDIKSKNVLLKNNLTACIADFGLALKFEAGKSAGDTHGQV
GTRRYMAPEVLEGAINFQRDAFLRIDMYAMGLVLWELASRCTAADGPVDEYMLPFEEEIG
QHPSLEDMQEVVVHKKKRPVLRDYWQKHAGMAMLCETIEECWDHDAEARLSAGCVGERIT
QMQRLTNIITTEDIVTVVTMVTNVDFPPKESSL (SEQ ID No. 11)

The terms "**ActRIIB** and "**ACVR2B**" as used herein refers to the human protein with UniProt ID Q13705.

MTAPWVALALLWGSLCAGSGRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCY
ASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAG
GPEVTYEPPPTAPTLLTVLAYSLLPIGGLSLIVLLAFWMYRHRKPPYGHVDIHEDPGPPP
PSPLVGLKPLQLLEIKARGRFGCVWKAQLMNDFVAVKIFPLQDKQSWQSEREIFSTPGMK
HENLLQFIAAEKRGSNLEVELWLITAFHDKGSLTDYLKGNIITWNELCHVAETMSRGLSY
LHEDVPWCRGEGHKPSIAHRDFKSKNVLLKSDLTAVLADFGLAVRFEPGKPPGDTHGQVG
TRRYMAPEVLEGAINFQRDAFLRIDMYAMGLVLWELVSRCKAADGPVDEYMLPFEEEIGQ
HPSLEELQEVVVHKKMRPTIKDHWLKHPGLAQLCVTIEECWDHDAEARLSAGCVEERVSL
IRRSVNGTTSDCLVSLVTSVTNVDLPPKESSI (SEQ ID No. 12)

Activin receptor II B (ActRIIB) is a receptor for myostatin, activin, and bone morphogenetic proteins (BMPs). The interaction between myostatin and this receptor regulates the inhibition of skeletal muscle differentiation via the Smad-dependent pathway. It is thought that by inhibiting or preventing myostatin from binding to ActRIIB, e.g, via an ActRII receptor antibody, the formation of skeletal muscle can be induced. Regulation of the activin receptor II A (ActRIIA) also plays a role in the regulation of muscle growth (Morvan et al. 2017).

Exemplary ActRII receptor antibodies that bind to ActRIIA and ActRIIB were shown to not only increase lean muscle mass, but also to decrease fat mass and improve glycemic control in human clinical studies (W02010/125003, WO2018/116201, Heymsfield et al. 2021;4(I):e2033457, JAMA). Exemplary ActRII receptor antibodies that may be used in the context of the present disclosure include the antibodies described in W02010/125003, WO2013/063536, WO2013/188448, WO2014/172448, WO2017/156488, WO2020/243448 and WO2021/174198.

In specific embodiments, the present disclosure relates to the antibody Bimagrumab (BYM338; W02010/125003). Bimagrumab has the following amino acid sequence.

Table 1:

SEQ ID No.	Description	Sequence
1	CDRH1 (Kabat)	SSYIN
2	CDRH2 (Kabat)	TINPVSGSTSYAQKFQG
3	CDRH3 (Kabat)	GGWFDY
4	CDRL1 (Kabat)	TGTSSDVGSYNYVN
5	CDRL2 (Kabat)	GVSKRPS
6	CDRL3 (Kabat)	GTFAGGSYYGV
7	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGL EWMGTINPVSGSTSYAQKFQGRVTMTRDTSISTAYMELSRLRSDD TAVYYCARGGWFDYWGQGTLVTVSS
8	VL	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKA PKLMIYGVSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYC GTFAGGSYYGVFGGGTKLTVLGQ
9	Full heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGL EWMGTINPVSGSTSYAQKFQGRVTMTRDTSISTAYMELSRLRSDD TAVYYCARGGWFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCP PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

10	Full light chain	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKA PKLMIYGVSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYC GTFAGGSYYGVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

In certain embodiments, the ActRII receptor antibody of the present disclosure comprises a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3).

In certain embodiments, the ActRII receptor antibody of the present disclosure comprises a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3).

In certain embodiments, the ActRII receptor antibody of the present disclosure comprises a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3).

In certain embodiments, the ActRII receptor antibody of the present disclosure comprises a variable heavy chain comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence of at least 80 percent, 81 percent, 82 percent, 83 percent, 84 percent, 85 percent, 86 percent, 87 percent, 88 percent, 89 percent, 90 percent, 91 percent, 92 percent, 93 percent, 94 percent, 95 percent, 96 percent, 97 percent, 98 percent, or 99 percent sequence identity thereto; and/or a variable light chain comprising the amino acid sequence of SEQ ID NO: 8, or an amino acid sequence of at least 90 percent, 91 percent, 92 percent, 93 percent, 94 percent, 95 percent, 96 percent, 97 percent, 98 percent, or 99 percent sequence identity thereto.

In certain embodiments, the ActRII receptor antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 9, or an amino acid sequence of at least 80 percent, 81 percent, 82 percent, 83 percent, 84 percent, 85 percent, 86 percent, 87 percent, 88 percent, 89 percent, 90 percent, 91 percent, 92 percent, 93 percent, 94 percent, 95 percent, 96 percent, 97 percent, 98 percent, or 99 percent sequence identity thereto; and/or a light chain comprising the amino acid sequence of SEQ ID NO: 10, or an amino acid sequence of at least 90 percent, 91 percent, 92 percent, 93 percent, 94 percent, 95 percent, 96 percent, 97 percent, 98 percent, or 99 percent sequence identity thereto.

In certain embodiments, the ActRII receptor antibody of the present disclosure has the same binding specificity as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3).

In certain embodiments, the ActRII receptor antibody of the present disclosure binds to the same epitope as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3).

In certain embodiments, the ActRII receptor antibody of the present disclosure binds to the same epitope as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3), and wherein said ActRII receptor antibody contains a engineered cysteine.

In certain embodiments, the ActRII receptor antibody of the present disclosure binds to the same epitope as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3), and wherein said ActRII receptor antibody contains a engineered cysteine at position 272, 361, 384, 389 or 434.

In certain embodiments, the ActRII receptor antibody of the present disclosure binds to the same epitope as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3), wherein said antibody comprises a silencing Fc modification. In certain embodiments, said silencing mutation is a LALA mutation.

In certain embodiments, the ActRII receptor antibody of the present disclosure binds to the same epitope as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3), wherein said antibody comprises a silencing Fc modification. In certain embodiments, said silencing mutation is a PA-LALA mutation.

In certain embodiments, the ActRII receptor antibody of the present disclosure binds to the same epitope as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3), wherein said antibody comprises a silencing Fc modification. In certain embodiments, said silencing mutation is a PG-LALA mutation.

In certain embodiments, the ActRII receptor antibody of the present disclosure binds to the same epitope as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3), wherein said antibody comprises a silencing Fc modification. In certain embodiments, said silencing mutation is a AEASS mutation.

In certain embodiments, the Fc part of the ActRII receptor antibody of the present disclosure may also be an allotype. One well known exemplary allotype is the R↔K allotype which, when introduced into the Fc region of bimagrumab, leads to the following amino acid sequence of the full heavy chain:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGTINPVSGSTSYAQKF
QGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGWFDYWGQGTLVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDK**K**VEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID No. 13)

The terms "**silent**", "**silenced**" as used herein in the context of a silenced antibody or an antibody comprising a silencing mutation refers to a mutation in the Fc domain of such antibody which decreases, partially or wholly, binding to one or more cell surface Fcgamma receptors, thereby reducing or dampening, and in some embodiments abrogating substantially completely, one or more Fc-mediated antibody effector functions, such as ADCC, ADCP, and CDC complement response (see, e.g., Kang and Jung, Experimental and Molecular Medicine (2019) 51:138). Silenced effector functions can be obtained by mutation in the Fc region of the antibody and have been described in the Art (e.g., Strohl, Biotechnology 20: 685-91 for LALA and N297A; Baudino et al., J. Immunol. 181: 6664-69 for D265A). Other exemplary Fc silencing mutations include amino acid substitutions at one of more of positions E233, L234, L235, G236, N297, P331 and P329 (see e.g. U.S. Pat. Nos.6,737,056, 7,332,581; WO 2004/056312, WO2021/234402, and Shields, R. L. et al., J. Biol. Chem.276 (2001) 6591-6604).

Silencing mutations also include (numbering according EU index) the LALA (L234A/L235A), the PA-LALA (L234A/L235A/P329A) and the PG-LALA (L234A/L235A/P329G) mutations, as well as the AEASS mutations (L234A/L235E/G237A/A330S/P331S).

In certain embodiments, the ActRII receptor antibody of the present disclosure is a modified or an improved version of an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3). Preferably, said modified or improved version of said antibody has a higher affinity to ActRIIA in order to obtain equal potent inhibition of both ActRII receptors. It shows less unspecific binding aiming to improve PK and in line with the PK, associated PD effects. It has optimized stability for tailor-made formulation.

In certain embodiments, the ActRII receptor antibody of the present disclosure has a Fc domain, for example a human IgG1 Fc domain, a human IgG2 Fc domain, a human IgG3, Fc domain, or a human IgG4 Fc domain. In certain embodiments, the Fc domain is wild type. In In certain embodiments, the ActRII receptor antibody of the present disclosure comprises embodiments, the Fc domain is modified, e.g. modified to increase serum half-life. In In certain embodiments, the ActRII receptor antibody of the present disclosure comprises embodiments, the Fc domain is a human IgG1 Fc domain, and modified to increase serum half-life. In In certain embodiments, the ActRII receptor antibody of the present disclosure comprises embodiments the modifications that increase serum half-life include one or more of 259I, 252Y, 307Q, 308F, 428L, 434H, 434F, 434Y, 434A, 434M, and 434S relative to a human IgG1 Fc domain according to the EU numbering scheme. In In certain embodiments, the ActRII receptor antibody of the present disclosure comprises embodiments, the modifications include one or more of 259I/434S, 308F/434S, 308F/428L/434S, 259I/308F/434S, 307Q/308F/434S, 250I/308F/434S, and 308F1319L/434S relative to a human IgG1 Fc domain according to the EU numbering scheme.

In certain embodiments, the ActRII receptor antibody of the present the Fc domain is modified to comprise the mutations M252Y, S254T and T256E relative to a human IgG1 Fc domain according to the EU numbering scheme. These mutation are referred to as "**YTE mutations**". Antibodies carrying a YTE mutation are reported to increase the half-life of the antibody (WO2002/060919)

In certain embodiments, the ActRII receptor antibody of the present disclosure binds to ActRIIB with a KD of 100 nM or less, 10 nM or less, 1 nM or less. Preferably, an ActRII receptor antibody binds to ActRIIB with an affinity of 100 pM or less (i.e. 100 pM, 50 pM, 10 pM, 1 pM or less). In certain embodiments, the ActRII receptor antibody binds to ActRIIB with an affinity of between 10 and 20 pM.

In certain embodiments, the ActRII receptor antibody of the present disclosure binds to ActRIIB with a 5 -fold greater affinity than to ActRIIA, more preferably 10-fold, still more preferably 50-fold, still more preferably 100-fold. In some embodiments, the ActRII receptor antibody binds to ActRIIA with an affinity of 100 pM or more (i.e. 250 pM, 500 pM, 1 nM, 5 nM or more).

In certain embodiments a ActRII pathway agent other than a ActRII receptor antibody is used. Such ActRII pathway agent binds an ActRII receptor ligand, e.g., myostatin or activin directly. Such agents include, but are not limited to, a myostatin inhibitor (e.g., a myostatin antibody, or a myostatin small molecule antagonist), an activin inhibitor (e.g., an activin antibody, or an activin small molecule antagonist), or a soluble extracellular portion of ActRIIB or ActRIIA that can act as a "ligand sink" optionally further stabilized with an Fc. Accordingly, in certain embodiments, provided herein are methods for treating a metabolic disorder of the disclosure, comprising administering to a subject in need a ActRII pathway agent (other than an ActRII receptor antibody) in combination with a GLP-1 agonist, wherein the agent is selected from the group consisting of a myostatin inhibitor, an activin inhibitor, or a soluble portion of an ActRII receptor.

GLP-1 agonists

The glucagon-like peptide- 1 receptor is mainly recognized as a pancreatic beta cell receptor that stimulates insulin secretion upon binding of the hormone glucagon-like peptide 1 (GLP-1). GLP-1 is a type of incretin hormone, all of which regulate insulin release in response to blood sugar. GLP-1 agonists mimic the action of GLP-1 peptide and activate the GLP-1 receptor upon binding, stimulating insulin secretion. Besides its glucose-regulatory effects GLP-1 has also been identified to act in other tissues such as the brain, stomach, intestine, heart and muscle.

However, GLP-1 agonists exhibit significant side effects and tolerability issues at effective doses. GLP-1 agonists have also been shown to decrease lean mass of a subject during treatment. Warnings and precautions for GLP-1 agonists (e.g., semaglutide) include but are not limited to: thyroid C-cell tumors, acute pancreatitis, acute gallbladder disease, hypoglycemia, acute kidney injury, severe adverse gastrointestinal reactions, hypersensitivity, e.g., anaphylactic reactions and angioedema, diabetic retinopathy complications in subjects Type 2 Diabetes, heart rate increase, and suicidal behavior and ideation. Adverse reactions reported in at least 5 percent of subjects treated with a GLP-1 agonist (e.g., semaglutide), include nausea, diarrhea, vomiting, constipation, abdominal pain, headache, fatigue, dyspepsia, dizziness, abdominal distension, eructation, hypoglycemia in patients

with type 2 diabetes, flatulence, gastroenteritis, and gastroesophageal reflux disease. Thus, there is a need for optimized dosage and treatments with GLP-1 agonists.

The term "**GLP-1 agonist**" as used herein refers to a compound, which fully or partially activates the human GLP-1 receptor. The term GLP-1 agonists also includes GLP-1 dual agonists which also activate the GIP-receptor or the Glucagon receptor, respectively, as well as GLP-1 triagonists which activate the GLP-1, GIP and GCG receptors simultaneously and to a varying extent.

As provided herein, the GLP-1 agonist of the disclosure may be a peptide, antibody, small molecule, or an aptamer. In some embodiments, the GLP-1 agonist is a peptide or peptide analog, including but not limited to: exenatide, exenatide extended-release, dulaglutide, liraglutide, lixisenatide, semaglutide, tirzepatide, cotadutide, noiiglutide, cotadutide, oxyntomodulin, or pemvidutide. In some embodiments, the GLP-1 agonist is a small molecule non-peptide agonist, e.g, danuglipron. GLP-1 agonists are also described and characterized in Obes Sci Pract. 2017 Mar; 3(1): 3–14, Ther Adv Endocrinol Metab 2021, Vol. 12: 1–15 and 2022; 23(3): 521–539.

Other exemplary GLP-1 agonists include:

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-K(MiniPEGAcBr) (SEQ ID No. 14)

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-KKgGluC16(MiniPEGAcBr) (SEQ ID No. 15)

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-K(GGGGS)3AcBr (SEQ ID No. 16)

HAibEGTFTSDVSSYLEGQAA-K(MiniPEGAcBr)-EFIAWLVRGRG (SEQ ID No. 17)

HAibEGTFTSDVSSYLEGQAA-KKgGluC16(MiniPEGAcBr)-EFIAWLVRGRG (SEQ ID No. 18)

HAibEGTFTSDVSSYLEGQAA-K(GGGGS)3AcBr-EFIAWLVRGRG (SEQ ID No. 19)

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-K(MiniPEGAcBr) (SEQ ID No. 20)

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-KKgGluC16(MiniPEGAcBr) (SEQ ID No. 21)

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-K(GGGGS)3AcBr (SEQ ID No. 22)

The term "**GLP-1 dual agonist**" as used herein refers to GLP-1 agonist which activates a second receptor in addition to a GLP-1 receptor, such as the glucose-dependent insulinotropic (GIP) receptor or the glucagon (GCG) receptor.

In certain embodiments, the GLP-1 agonist is a dual agonist that binds and activates or deactivates a second receptor in addition to a GLP-1 receptor, wherein the second receptor is glucose-dependent insulinotropic (GIP) receptor or a glucagon (GCG) receptor. An exemplary dual acting GLP-1 agonist that binds both GLP-1 receptor and GIP receptor is tirzepatide. Exemplary dual acting GLP-1 agonists that binds both GLP-1 receptor and GCG receptor are cotadutide, noiiglutide, and oxyntomodulin. In some embodiments, the GLP-1 agonist is also a GIP antagonist. In some embodiments, the GLP-1 agonist is also a GCG agonist.

Exemplary GLP-1/GIP dual agonists include:

YAibEGTFTSDYSIYLDKQAAAibEFVNWLLAGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 23)

YAibEGTFTSDYSIYLDKQAAAibEFV-K(MiniPEGAcBr)-WLLAGGPSSGAPPPSK-NH2 (SEQ ID No. 24)

YAibEGTFTSDYSIYLDKQAAAibEFVNWLLAG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 25)

YAibEGTFTSDYSIYLDKQAAAibEFV-K(MiniPEGAcBr)-WLLAG-NH2 (SEQ ID No. 26)

GLP-1/GIP dual agonists are also described and characterized in Diabetes Metab Syndr Obes 2019; 12: 1973–198, Bioorg Chem. 2021; 106:104492 and Drug Design, Development and Therapy 2022:16 1547–1559.

Exemplary GLP-1/GCG dual agonists include:

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 27)

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 28)

YAibAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 29)

YAibQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 30)

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSarGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 31)

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSar-K(MiniPEGAcBr)-NH2 (SEQ ID No. 32)

YAibAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSarGPSSGAPPPSK(MiniPEGAcBr)-NH2 (SEQ ID No. 33)

YAibQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSar-K(MiniPEGAcBr)-NH2 (SEQ ID No. 34)

GLP-1/GCG dual agonists are also described and characterized in Diabetologia (2017) 60(10):1851–61, Bioconjugate Chem. 2020, 31, 4, 1167–1176 and Nature Communications (2022) 13:3613.

The term "**GLP-1 triagonist**" as used herein refers to GLP-1 agonist which activates two receptors in addition to a GLP-1 receptor, such as the glucose-dependent insulinotropic (GIP) receptor and the glucagon (GCG) receptor.

In certain embodiments, the GLP-1 agonist is a GLP-1 triagonist. In certain embodiments, the GLP-1 agonist is a triagonist of GIP/GLP/glucagon receptor, e.g., a GGG triagonist, e.g., LY343794.

Exemplary GLP-1/GIP/GCG triple agonists include:

Ac-hAQGTFTSD-K(MiniPEGAcBr)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-NH2 (SEQ ID No. 35)

Ac-hAQGTFTSDKSKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 36)

Ac-hAQGTFTSD-K(MiniPEGAcBr)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(gGluC16)-NH2 (SEQ ID No. 37)

Ac-hAQGTFTSD-K(gGluC16)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 38)

GLP-1 triagonists are also described and characterized in Nat Med 2015; 21(1):27-36, Endocrine Reviews, 2018, 39(5):719-38 and Cell Metabolism 34, 1234–1247, September 6, 2022.

Abbreviations and structures that are used in the context of the GLP-1 peptides:

h: D-Histidine

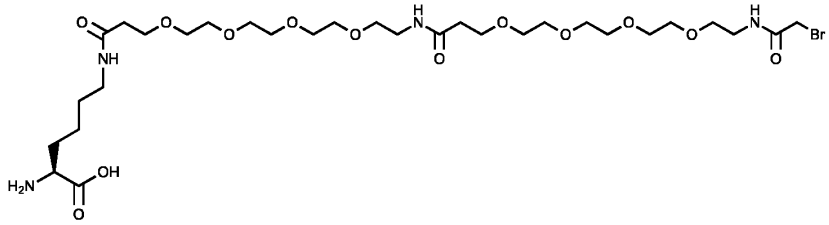
βAla: β-Alanine

Aib: 2-Amino isobutyric acid

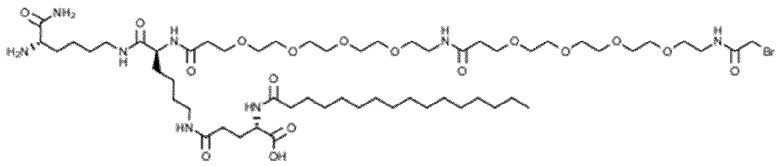
isoAsp: isoaspartic acid

Sar: Sarcosine

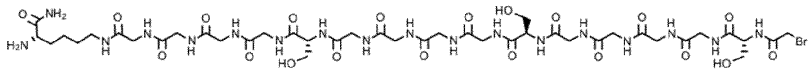
K(MiniPEGAcBr):



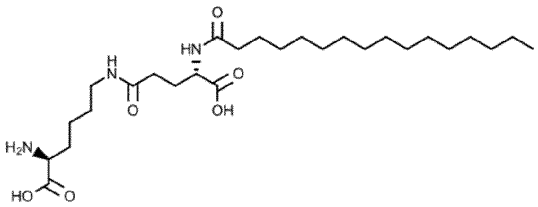
KKγGluC16MiniPEGAcBr:



K(GGGGS)3AcBr:



KγGluC16:



Conjugation

Various technologies exist, by which peptides or modified peptides can be conjugated to antibodies. This includes cysteine engineering of antibodies. In this technology peptides are conjugate to cysteines that are artificially introduced into the antibody, typically the Fc part.

The term "**cysteine engineered**" or "**engineered cysteine**" as used herein in the context of antibodies, refers to an antibody construct that is engineered to introduce at least one cysteine insertion mutation. A "cysteine mutation" in this context refers to a non-native cysteine residue that is introduced into the parental antibody construct sequence. The inserted cysteine residue(s) may be used as a site for conjugation of one or more active agents. In the present disclosure such active agent is an agonistic GLP-1 peptide.

Various position in antibodies were reported as being amenable for cysteine engineering, including but not limited to positions 241, 243, 251, 253, 258, 264, 269, 271, 272, 274, 280, 281, 285, 288, 291, 293, 294, 296, 301, 307, 309, 311, 318, 329, 340, 341, 345, 357, 361, 384, 385, 386, 387, 389, 401, 402, 411, 417, 433, 434, 435, and 439 (from Medimmune_WO2015157595). Exemplified herein are antibodies that are cysteine engineered are positions E272, E361, N384, E389 and N434.

Peptides can be coupled to the cysteine engineered antibodies via Michael addition to, e.g., maleimide-modified peptides, or via halogen-substitution of accordingly modified peptides, e.g. Bromo acetylated peptides. Chemically modified peptides can for example be conjugated to cysteine engineered antibodies as described in ACS Chem Biol. 2017 Sep 15;12(9):2427-2435.

Other conjugation technologies are known as well that can be used to generate a conjugate comprising an ActRII receptor antibody and an agonistic GLP-1 peptide. Such additional conjugation technologies include conjugation via lysine-linked peptides or acetylene/azide modified peptides (via click chemistry).

In certain embodiments, the present disclosure relates to a immunoconjugate comprising an ActRII receptor antibody and an agonistic GLP-1 peptide, wherein said ActRII receptor antibody is conjugated to said agonistic GLP-1 peptide via cysteine engineering.

In certain embodiments, the present disclosure relates to a immunoconjugate comprising an ActRII receptor antibody and an agonistic GLP-1 peptide, wherein said ActRII receptor antibody is conjugated to said agonistic GLP-1 peptide via cysteine engineering, and wherein said conjugation technology employs bromo acetylated peptides.

In certain embodiments, the present disclosure relates to a immunoconjugate comprising an ActRII receptor antibody and an agonistic GLP-1 peptide, wherein said agonistic GLP-1 peptide is conjugated to said ActRII receptor antibody at position 272, 361, 384, 389 or 434 of the ActRII receptor antibody. Said agonistic GLP-1 peptide may also be conjugated to said ActRII receptor antibody via a cysteine residue located in the hinge region.

Furthermore, spacers of various length and rigidity may be introduced to further optimize the immunoconjugates of the present disclosure. Commonly used spacers include polyamides or polyethylene glycol (PEG).

ActRII antagonists

In certain embodiments, the ActRII receptor antibody of the immunoconjugates disclosed herein may be replaced by an ActRII antagonist. Such ActRII antagonist may be a moiety different than an ActRII receptor antibody, for example a TGFβ-like ligand, such as myosatin, AT, propeptide, activin A or derivatives or analogs thereof. Preferably said Actrll antagonist is an antagonist of ActRIIA and ActRIIB.

Therefore, in certain embodiment the present disclosure relates to a conjugate comprising an ActRII antagonist and an agonistic GLP-1 peptide.

In certain embodiments, the present disclosure relates to a conjugate comprising an ActRII antagonist and an agonistic GLP-1 peptide, wherein said ActRII antagonist is specific for a polypeptide of SEQ ID No. 12 and a polypeptide of SEQ ID No. 11.

In certain embodiments, the present disclosure relates to a conjugate comprising an ActRII antagonist and an agonistic GLP-1 peptide, wherein said agonistic GLP-1 peptide is selected from

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-K(MiniPEGAcBr) (SEQ ID No. 14),

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-KKgGluC16(MiniPEGAcBr) (SEQ ID No. 15),

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-K(GGGGS)3AcBr (SEQ ID No. 16),

HAibEGTFTSDVSSYLEGQAA-K(MiniPEGAcBr)-EFIAWLVRGRG (SEQ ID No. 17),

HAibEGTFTSDVSSYLEGQAA-KKgGluC16(MiniPEGAcBr)-EFIAWLVRGRG (SEQ ID No. 18),

HAibEGTFTSDVSSYLEGQAA-K(GGGGS)3AcBr-EFIAWLVRGRG (SEQ ID No. 19),

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-K(MiniPEGAcBr) (SEQ ID No. 20),

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-KKgGluC16(MiniPEGAcBr) (SEQ ID No. 21), and

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-K(GGGGS)3AcBr (SEQ ID No. 22).

In certain embodiments, the present disclosure relates to a conjugate comprising an ActRII antagonist and an agonistic GLP-1 peptide, wherein said agonistic GLP-1 peptide is GLP-1 dual agonist or a GLP-1 triagonist. Preferably said GLP-1 dual agonist or a GLP-1 triagonist is selected from

YAibEGTFTSDYSIYLDKQAAAibEFVNWLLAGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 23),

YAibEGTFTSDYSIYLDKQAAAibEFV-K(MiniPEGAcBr)-WLLAGGPSSGAPPPSK-NH2 (SEQ ID No. 24),

YAibEGTFTSDYSIYLDKQAAAibEFVNWLLAG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 25),

YAibEGTFTSDYSIYLDKQAAAibEFV-K(MiniPEGAcBr)-WLLAG-NH2 (SEQ ID No. 26),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 27),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 28),

YAibAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 29),

YAibQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 30),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSarGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 31),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSar-K(MiniPEGAcBr)-NH2 (SEQ ID No. 32),

YAibAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSarGPSSGAPPPSK(MiniPEGAcBr)-NH2 (SEQ ID No. 33),

YAibQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSar-K(MiniPEGAcBr)-NH2 (SEQ ID No. 34),

Ac-hAQGTFTSD-K(MiniPEGAcBr)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-NH2 (SEQ ID No. 35),

Ac-hAQGTFTSDKSKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 36),

Ac-hAQGTFTSD-K(MiniPEGAcBr)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(gGluC16)-NH2 (SEQ ID No. 37), and

Ac-hAQGTFTSD-K(gGluC16)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 38).

GLP-1 agonists

In certain embodiments, the agonistic GLP-1 peptide of the immunoconjugates disclosed herein may be replaced by an agonistic GLP-1 small molecule. Such small molecules are often referred to as GLP-1 secretagogues (Drug Disc Today 25, 2023 (2021); Nat Commun 13, 4633 (2022). This term refers to an agent that promotes GLP-1 secretion from a cell, e.g. an enteroendocrine cell. Exemplary GLP-1 secretagogues include BDM72881, HD0471042, YH1896, BMS-903452, LY292208, LY2922470, BMs9-86118, SCO-267 and JWU-A021.

Therefore, in certain embodiment the present disclosure relates to a conjugate comprising an ActRII receptor antibody and an agonistic GLP-1 small molecule. In certain embodiment, the present disclosure relates to a conjugate comprising an ActRII receptor antibody and a GLP-1 secretagogue. In certain embodiments said agonistic GLP-1 small molecule or GLP-1 secretagogue is selected from BDM72881, HD0471042, YH1896, BMS-903452, LY292208, LY2922470, BMs9-86118, SCO-267 and JWU-A021.

In certain embodiment, the present disclosure relates to a conjugate comprising an ActRII receptor antibody and an agonistic GLP-1 small molecule, wherein said ActRII receptor antibody is specific for a polypeptide of SEQ ID No. 12 and for a polypeptide of SEQ ID No. 11. In certain embodiment, the present disclosure relates to a conjugate comprising an ActRII receptor antibody and a GLP-1 secretagogue, wherein said ActRII receptor antibody is specific for a polypeptide of SEQ ID No. 12 and for a polypeptide of SEQ ID No. 11.

In certain embodiment, the present disclosure relates to a conjugate comprising an ActRII receptor antibody and an agonistic GLP-1 small molecule, wherein said ActRII receptor antibody is binds to the same epitope as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3). In certain embodiment, the present disclosure relates to a conjugate comprising an ActRII receptor antibody and a GLP-1 secretagogue, wherein said ActRII receptor antibody is binds to the same epitope as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3).

Antibody fusion

The agonistic GLP-1 peptides of the present disclosure may also be genetically fused to the ActRII receptor antibodies. Preferably, said agonistic GLP-1 peptides are fused to the N-terminus of the variable heavy chain of the N-terminus of the variable light. Optionally the agonistic GLP-1 peptide and the variable heavy chain or the variable light chain of the ActRII antibody are separated by a linker. The agonistic GLP-1 peptide may be fused either to one or to two variable chains of the antibody. If the agonistic GLP-1 peptides are fused to two variable chains of the antibody, then both agonistic GLP-1 peptides are either fused to the variable heavy chain or both agonistic GLP-1 peptides are fused to the variable light chain.

Therefore, in certain embodiments the present disclosure relates to an ActRII receptor antibody, wherein said ActRII receptor antibody is fused to an agonistic GLP-1 peptide. In certain embodiments, the present disclosure relates to an ActRII receptor antibody, wherein said ActRII receptor antibody is genetically fused to an agonistic GLP-1 peptide.

In certain embodiments, the present disclosure relates to an ActRII receptor antibody, wherein said ActRII receptor antibody is fused to an agonistic GLP-1 peptide and wherein said agonistic GLP-1 peptides is fused to the N-terminus of the variable heavy chain of the ActRII receptor antibody. In certain embodiments, the present disclosure relates to an ActRII receptor antibody, wherein said ActRII receptor antibody is fused to an agonistic GLP-1 peptide and wherein said agonistic GLP-1 peptides is fused to the N-terminus of the variable light chain of the ActRII receptor antibody.

In certain embodiments, the present disclosure relates to an ActRII receptor antibody, wherein said ActRII receptor antibody is fused to an agonistic GLP-1 peptide, wherein said agonistic GLP-1 peptides is fused to the N-terminus of the variable heavy chain of the ActRII receptor antibody and wherein said agonistic GLP-1 peptide and said variable heavy chain are separated by a linker. In certain embodiments, the present disclosure relates to an ActRII receptor antibody, wherein said ActRII receptor antibody is fused to an agonistic GLP-1 peptide, wherein said agonistic GLP-1 peptides is fused to the N-terminus of the variable light chain of the ActRII receptor antibody, and wherein said agonistic GLP-1 peptide and variable light chain are separated by a linker. Preferably said linker is a glycine-serin rich linker. More preferably said linker is a $(G4S)_2$ linker. Also preferably said linker comprises or consists of the amino acid sequence of SEQ ID No. 48.

In certain embodiments, the present disclosure relates to an ActRII receptor antibody, wherein said ActRII receptor antibody is fused to one or to two variable chains of the ActRII receptor antibody.

In certain embodiments, the present disclosure relates to an ActRII receptor antibody, wherein said ActRII receptor antibody is fused to one variable chains of the ActRII receptor antibody. In certain embodiments said variable chain is the variable heavy chain. In other embodiments said variable chain is the variable light chain.

In certain embodiments, the present disclosure relates to an ActRII receptor antibody, wherein said ActRII receptor antibody is fused to both variable heavy chains of the ActRII receptor antibody. In other embodiments, the present disclosure relates to an ActRII receptor antibody, wherein said ActRII receptor antibody is fused to both variable light chains of the ActRII receptor antibody.

Treatment

The terms "**treatment**", "**treating**" and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect with a therapeutic agent. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof, e.g., reducing

the likelihood that the disease or symptom thereof occurs in the subject, and/or may be therapeutic in terms of completely or partially reducing a symptom, or a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a mammal, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting or slowing the onset or development of the disease; or (c) relieving the disease, e.g., causing regression of the disease or symptoms associated with the disease. The therapeutic agent may be administered before, during or after the onset of disease. The treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, may be of particular interest. In some embodiments, treatment is performed prior to complete loss of function in the affected tissues. In some embodiments, the subject therapy will be administered during the symptomatic stage of the disease, and in some embodiments, after the symptomatic stage of the disease.

The term "**individual**", "**subject**" and "**patient**" used interchangeably herein and refer to any subject for whom treatment or therapy is desired. The subject may be a mammalian subject. Mammalian subjects include, e. g., humans, non-human primates, rodents, (e.g., rats, mice), lagomorphs (e.g, rabbits), ungulates (e.g., cows, sheep, pigs, horses, goats, and the like), etc. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human primate, for example a cynomolgus monkey. In some embodiments, the subject is a companion animal (e.g, cats, dogs).

The terms "**obesity related co-morbidity**," "**obesity related condition**" and "**obesity related disorder**" may be used interchangeably and refer to a health condition depending from the obesity of the subject. In some embodiments, the obesity related co-morbidity or condition increases the mortality risk of the subject. Obesity related co-morbidities include but are not limited to: high blood pressure (hypertension), high LDL cholesterol, low HDL cholesterol, high levels of triglycerides (dyslipidemia), Type 2 diabetes, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea, breathing problems, cancer, gastroesophageal reflux disease, severe COVID-19, overall mortality, lower quality of life, mental illness such as clinical depression, anxiety, and other mental disorders, and body pain and difficulty with physical functioning.

A "**metabolic disorder**" as used herein refers to a disorder affecting dysregulation of mammalian metabolism, including but not limited to: obesity, diabetes (Type I and II), metabolic syndrome, antipsychotic drug-associated obesity, glucocorticoid-induced obesity, hypothalamic obesity associated with craniopharyngioma, and monogenetic disorder associated obesity. The monogenetic disorders associated with obesity in humans, may include but are not limited to Bardet-Biedl syndrome, and a disorder arising from a mutation in one or more of the following genes ADCY3, ALMS1, ARL6, BBS1, BBS2, BBS4, BBS5, BBS7, BBS9, BBS10, BBS12, BDNF, CCDC28B, CEP290, CREBBP, EP300, GNAS, IER3IP1,

MC3R, MKKS, MKS1, MRAP2, NTRK2, PCSK1, PHF6, POMC, SH2B1, SIM1, TMEM67, TRIM32, TTC8 and VPS13B, or combinations thereof. A metabolic disorder may also be associated with a complex genetic disorder, e.g., Prader-Willi syndrome.

As used herein, "**Body Mass Index**" or "**BMI**" is calculated as weight in kilograms (kg) divided by height in meters squared (m^2), rounded to one decimal place. As used herein, "obesity" in adult humans is defined as a BMI greater than or equal to 30 kg/m^2. "**Obesity**" in human youth is defined as a BMI greater than or equal to the age- and sex-specific 95th percentile of the 2000 CDC growth charts. The term "**overweight** " is defined as a BMI of greater than or equal to 25 and less than 30.

"**Lean mass**" is defined as total body mass of a subject minus the fat mass and minus the bone mass of the subject. Lean mass and fat mass may be measured by, for example, bioelectrical impedance analysis (BIA), magnetic resonance imaging (MRI) or dual X-ray absorptiometry (DXA).

The term "**pharmaceutical composition**" as used herein refers to a pharmaceutical active compounds, such as the immunoconjugates of the present disclosure, and a pharmaceutically acceptable excipient.

In certain embodiments, the present disclosure relates to a pharmaceutical composition comprising an immune conjugate of the present disclosure.

In certain embodiments, the present disclosure relates to an immunoconjugate or a pharmaceutical composition of the present disclosure for use in medicine. In certain embodiments, said use in medicine is the use in the treatment of a metabolic disorder. In certain embodiments, said metabolic disorder is selected from the group consisting of: obesity, diabetes, metabolic syndrome, anti-psychotic drug-associated obesity, glucocorticoid-induced obesity, hypothalamic obesity associated with craniopharyngioma, Prader-Willi syndrome, and a monogenetic disorder associated with obesity. In certain embodiments, said treatment is useful for an obesity related co-morbidity, wherein the condition is selected from the group of: glucose intolerance, prediabetes, insulin resistance, high triglycerides, overweight associated physical impairment, osteoporosis, renal disease, obstructive sleep apnea, sexual hormones impairment, endocrine reproductive disorders, osteoarthritis, gastrointestinal cancers, dyslipidemia, hypertension, heart failure, coronary heart disease, stroke, and/or gallstones.

Examples

Example 1: Generation of antibodies

Several ActRII receptor antibodies were produced. Basis for the constructs was the antibody bimagrumab, i.e. an antibody comprising the variable heavy chain of SEQ ID No. 7 and the variable light chain of SEQ ID No. 8. Various derivatives of bimagrumab were utilized in the present study, all of which do not affect the antibody specificity.

For the conjugation of GLP-1 peptides to the antibody, cysteine engineered versions of bimagrumab were generated by substitution of various amino acids in the Fc region to a cysteine. Specifically the following cysteine engineered variants were generated: E272C, N384C, N434C, N361C and N389C. The respective antibodies have the following full heavy chain sequences:

Table 2:

Antibody	SEQ ID No.	Sequence
E272C	39	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLE WMGTINPVSGSTSYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTA VYYCARGGWFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKXVEPKSCDKTHTCPPCPAP EAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPCVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALAAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK, wherein X is K or R
N384C	40	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLE WMGTINPVSGSTSYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTA VYYCARGGWFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKXVEPKSCDKTHTCPPCPAP EAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALAAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESCGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK, wherein X is K or R

N434C	41	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLE WMGTINPVSGSTSYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTA VYYCARGGWFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKXVEPKSCDKTHTCPPCPAP EAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALAAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHCHYTQKSLSLSPGK, wherein X is K or R
N361C	42	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLE WMGTINPVSGSTSYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTA VYYCARGGWFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKXVEPKSCDKTHTCPPCPAP EAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALAAPIEKTISKAKGQPREPQVYTLPPSREEMTKCQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK, wherein X is K or R
N389C	43	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLE WMGTINPVSGSTSYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTA VYYCARGGWFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKXVEPKSCDKTHTCPPCPAP EAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALAAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPECNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK, wherein X is K or R

Principally, the antibodies may contain additional mutations, such as LALA, PA-LALA or PG-LALA silencing mutations in the Fc region or a YTE mutation to increase the half-life of the antibody.

Antibodies of the present disclosure were generated by Icosagen (Tartumaa, Estonia) using standard technologies. Binders were purified via HiTrap MabSelect PrismA and preparative SEC-HPLC. QC was performed using capillary electrophoresis on LabChip GXII, measurement of the endotoxin content and analytical SEC-HPLC. An exemplary capillary electrophoresis gel is shown in Figure 1. Endotoxin levels were below 0.02 EU/mg. Monomer content was >95 after one freeze-thaw cycle.

Example 2: Generation of agonistic GLP-1 peptides

Peptides are generated using Fmoc-strategy. All peptides are synthesized and purified on state-of-the-art instrumentation using Rink-resin as a solid support as exemplified for peptide 1.

Peptide 1

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-K(MiniPEG)AcBr (SEQ ID No. 14)

General procedure for peptide synthesis:

1) Resin preparation: The DMF (20 mL) was added to the Rink amide MBHA Resin (0.50 mmol, 1.00 eq, Sub: 0.5 mmol/g) and agitated under N_2 atmosphere at 25 °C for 0.5 hr. Then the reaction mixture was filtered off and the resin washed with DMF (40.0 mL * 5).
2) Deprotection Fmoc group: 20% piperidine in DMF (40.0 mL) was added to the resin and agitated under N_2 atmosphere at 25 °C for 15 min. The resin was washed with DMF (40.0 mL * 5) and filtered off.
3) Coupling: A solution of Fmoc-Lys(Dde)-OH (3.0 eq) and DIEA (6.0 eq) in DMF (20.0 mL) was added to the resin, then the HBTU (2.85 eq) was added, and the mixture was agitated under N_2 atmosphere at 25 °C for 30 min. The resin was washed with DMF (40.0 mL * 5). The coupling reaction was monitored by ninhydrin color reaction.
4) Repeat above step 2 to 3 for the coupling of following amino acids: (2-30).

Table 3:

#	Materials	Coupling reagents
1	Fmoc-Lys(Dde)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
2	Fmoc-Gly-Gly-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Lys(Boc)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-Val-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)

5	Fmoc-Leu-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
6	Fmoc-Trp(Boc)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
7	Fmoc-Ala-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
8	Fmoc-Ile-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
9	Fmoc-Phe-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
10	Fmoc-Glu(OtBu)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
11	Fmoc-Lys(Boc)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
12	Fmoc-Ala-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
13	Fmoc-Ala-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
14	Fmoc-Gln(Trt)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
15	Fmoc-Glu(OtBu)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
16	Fmoc-Glu(OtBu)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
17	Fmoc-Leu-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
18	Fmoc-Tyr(tBu)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
19	Fmoc-Ser(tBu)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
20	Fmoc-Ser(tBu)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
21	Fmoc-Val-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
22	Fmoc-Asp(OtBu)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
23	Fmoc-Ser(tBu)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
24	Fmoc-Thr(tBu)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
25	Fmoc-Phe-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
26	Fmoc-Thr(tBu)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
27	Fmoc-Gly-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
28	Fmoc-Glu(OtBu)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)

29	Fmoc-Gly-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
30	Boc-His(Trt)-OH (3.0 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)

5) Deprotection Dde group: 3% hydrazine hydrate in DMF (40.0 mL) was added and the resin agitated under N_2 atmosphere at 25 °C for 30 min. The resin was washed with DMF (40.0 mL * 5) and filtered off.
6) Coupling: A solution of Fmoc-PEG4-OH (3.0 eq) (3.0 eq) and DIEA (6.00 eq) in DMF (20.0 mL) was added to the resin, then the HBTU (2.85 eq) was added and the mixture agitated under N_2 atmosphere at 25 °C for 30 min. The resin was washed with DMF (40.0 mL * 5). The coupling reaction was monitored by ninhydrin color reaction.
7) Repeat above step 2 to 3 for the coupling of following amino acids: (32).

Table 4:

31	Fmoc-PEG4-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
32	Fmoc-PEG4-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)

8) Deprotection Fmoc group: 20% piperidine in DMF (40.0 mL) was added and the resin agitated under N_2 atmosphere at 25 °C for 15 min. The resin was washed with DMF (40.0 mL * 5) and filtered off.
9) Coupling AcBr: A solution of Bromoacetic acid (6.0 eq) and DIC (6.00 eq) in DMF (20.0 mL) was added to the resin and agitated under N_2 atmosphere at 25 °C for 30 min. The resin was washed with DMF (40.0 mL * 5). The coupling reaction was monitored by ninhydrin color reaction.

Peptide Cleavage and Purification:

1) The resin was washed with DMF (50 mL) two times, washed with MeOH (50 mL) three times and dried under vacuum to get 5.0 g of peptide resin.
2) 50 mL cleavage cocktail (2.5%H2O/2.5%Tis/2.5%MPA/92.5%TFA) was added to the flask containing the side chain protected peptide resin at 25 °C and the mixture was stirred for 120 min.

3) The cleavage cocktail was collected after filtration, the crude peptide was precipitated with 500 mL cold isopropyl ether and centrifuged (2 min at 3000 rpm) and washed two times with 500 mL isopropyl ether. The crude peptide was dried under vacuum for 1 hr.
4) The crude peptide (2.0 g) was purified by prep-HPLC (TFA condition: A: 0.075 % TFA in H_2O, B: ACN) to get the final product (217.8 mg, 9.64% yield, 89.77% purity, TFA) as a white solid which was confirmed by LCMS.

Purification conditions

Table 5:

Separation condition	
Dissolution condition	Dissolve in 80%TFA-H2O
Instrument	Gilson GX-281
Mobile Phase	A: H_2O (0.075% TFA in H_2O)
	B: CH_3CN
Gradient	23-48-50 min, Retention time: 23.5 min
Column	luna ,10um, c18,100A, 25mm+Gemin,5um,c18,110A
Flow Rate	20 mL/Min
Wavelength	220/254 nm
Oven Tem.	30°C

Analytical data:

R_t= 11.442 min, purity: 89.77%, MS cal.: 4057.32, MS observed: $[M+3H]^{3+}$ = 1353.3156

The following peptides were synthesized accordingly:

Peptide 2:

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-K(GGGGS)3AcBr (SEQ ID No. 16)

R_t= 13.3 min, purity: 91.58%, MS cal.: 4508.6, MS observed: $[M+3H]^{3+}$ = 1503.67

Peptide 3:

HAibEGTFTSDVSSYLEGQAA-K(MiniPEG)AcBr-EFIAWLVRGRG (SEQ ID No. 17)

R_t= 11.4 min, purity: 96.0%, MS cal.: 4012.29, MS observed: $[M+3H]^{3+}$ = 1338.31

Peptide 4:

HAibEGTFTSDVSSYLEGQAA-K(GGGGS)3AcBr-EFIAWLVRGRG (SEQ ID No. 19)

R_t= 10.2 min, purity: 93.25%, MS cal.: 4463.57, MS observed: $[M+3H]^{3+}$ = 1488.67

Peptide 5:

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-K(MiniPEG)AcBr (SEQ ID No. 20)

R_t= 10.8 min, purity: 87.11%, MS cal.: 4140.47, MS observed: $[M+3H]^{3+}$ = 1381.00

Peptide 6:

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-K(GGGGS)3AcBr (SEQ ID No. 22)

R_t= 12.9 min, purity: 95.71%, MS cal.: 4591.74, MS observed: $[M+3H]^{3+}$ = 1148.77

Abbreviations:

Aib: Aminoisobutyric acid

AcBr: Bromoacetic acid

MiniPEG: Amino-Polyethylene Glycol-Acid or 1-Amino-3,6,9,12-tetraoxapentadecan-15-oic acid – CAS 581065-95-4

MBHA Resin: 4-Methylbenzhydrylaminhydrochloride

DDE: 2-Acetyldimedone

MPA: 3-Mercaptopropionic acid

DIC: N,N'-Diisopropylcarbodiimid

TIS: Triisopropylsilane

Example 3: Conjugation of peptides to cysteine engineered antibodies

Peptides were coupled to the cysteine engineered antibodies via halogen-substitution of accordingly modified peptides, i.e. Bromo acetylated peptides. 5mg of the antibody dissolved in Histidine/NaCl buffer (conc 5mg/ml) was buffer exchanged to the same volume of reduction buffer

(50mM Na2SO4/2.0mM EDTA, pH7.5) via 3 cycles on Amicon Ultra-4, Centrifual Filters, Ultracel - 30K (3x 10min, 10000rpm). To the final volume 100eq of TCEP solution in water (0.5M conc) was added and the reaction vessel was shaken at 20° C for 22h. After the mix was desalted into new reaction buffer using Zeba desalt columns (MWCO 40000, prepared for use according to the buffer exchange procedure (Thermo Scientific)), using conjugation buffer (centrifuge to remove storage buffer 2 min, add 2 ml reaction buffer, centrifuge 2min, repeat 2x, centrifuge 6 min after 3rd buffer addition - centrifuge speed 700 g). Sample was then added to the desalt column and centrifuged for 4 minutes to collect the desalted sample. The eluent was then transferred into a 2ml Eppendorf tube and 20eq DHAA solution in PBS (10mM conc) was added. The solution was slightly shaken at RT. After another 22h 10eq BrAc modified peptide solution (10mM in Water) was added and the mixture was shaken at RT over night. Thereafter the sample was filter-purified and buffer exchanged to PBS buffer via 3 cycles on Amicon Ultra-4, Centrifual Filters, Ultracel - 10K (3x 10min, 10000rpm). The obtained conjugate in PBS (conc ca 5mg/ml) was then purified by prep SEC (Superdex 200 Increase, 10/300 GL column, PBS as eluent) and after pooling the pure fractions and the final analysis of the product was done by HIC-chromatography and LCMS. Final concentration was determined by Nanodrop UV. All immunoconjugates were characterized and quality assessed (MS, SDS PAGE, SEC, HIC, Heparin binding columns).

Example 4: ActR2A/B binding & functional activity

Example 4.1: Affinity of antibody & conjugates to ActRIIA and ActRIIB

The affinity interaction profile of the immunoglobulins was determined using an immuno-based-assay called Meso Scale Discovery (MSD) Solution Equilibrium Titration (MSD-SET). In this assay, the antigen (either ActRIIA or ActRIIB) was coated onto an MSD 96-well detection plate. The SET plate was prepared by mixing the immunoglobulins at a constant concentration with titrated antigen and incubated overnight at 4°C to reach equilibrium. After blocking of the MSD plate, 50 µl of the sample solutions containing the Immunoglobulin-Antigen mix (SET plate) were transferred onto the antigen-coated detection MSD plate. These samples were then incubated for 20 minutes to allow for binding of any free antibodies without significantly shifting the equilibrium. To detect the bound antibodies on the plate, a detection antibody Anti Human Antibody (Goat) Sulfo-TAG Labelled (PN: R32AJ-5) was added. The electrochemiluminescence (ECL) readout was then detected by adding a Read Buffer and measuring ECL using an MSD instrument (Sector S 600MM). Data analysis was performed on GraphPad Prism using equation for IgG from Journal of Biomolecular Screening 2015, Vol. 20(10) 1256–1267.

Tested in this experiment were various bimagrumab-derived antibodies, containing an engineered cysteine at position 272 (E272C). Antibodies were conjugated to peptides 5 and 6, and binding to receptors ActRIIA and ActRIIB was compared to the unconjugated cysteine-engineered antibody.

Results are shown in Table 6 and Figure 2. It could be demonstrated that binding of the antibodies to ActRIIA and ActRIIB is essentially unaffected by the conjugated peptide.

Table 6:

Antibody	ActRIIA [pM]	ActRIIB [pM]
E272C mAb	29	38
E272C mAb-peptide 5	40	33
E272C mAb-peptide 6	59	38

Example 4.2: Antagonistic activity in ActRIIA and B reporter cell assays

Furthermore, antagonistic activity was assessed in ActRIIA and B reporter cell assays assay. TGFB/SMAD Signaling Pathway SBE Reporter HEK293 cells (BPS BioScience) were washed with PBS, detached using 0.05% Trypsin and seeded at 35000 cells/well in a 96-well white flat-bottom plate in 100 µL Medium (MEM media, 10% FBS, 1% NEAA, 1 mM Na pyruvate, 1% Pen/Strep). The next day, medium was exchanged with 90 µL assay medium (MEM media, 0.5% FBS, 1% NEAA, 1 mM Na pyruvate, 1% Pen/Strep) containing diluted Antibodies of interest (10-0.00015 ug/mL) or Control. Cells were incubated at 37°C, 5% CO2 for 4h. Subsequently, 10 µL of 10ng/mL Activin (Cat#120-14E, Peprotech) or 500 ng/mL Myostatin (Cat#120-00, Peprotech) were added to the cells and cells incubated overnight. The next day, 100 µL of ONE-Step Luciferase Detection reagent (BPS Bioscience) were added to the cell solution (both equilibrated to room temperature) and incubated for 15 min on a plate shaker, before measuring luminescence using the Envision Plate Reader.

Tested in this experiment were various bimagrumab-derived antibodies, containing an engineered cysteine at position 272 (E272C). Antibodies were conjugated to various peptides as indicated below, and activity in ActRIIA and B reporter cell assays was compared to the unconjugated cysteine-engineered antibody.

Results are shown in the following Table 7 and Figure 3. It could be demonstrated that all tested conjugates remain active when measured either against Activin A or Myostatin.

Table 7:

Antibody	EC50 (pM) Activin	EC50 (pM) Myostatin
E272C mAb	423	nd
E272C mAb-peptide 1	986	nd
E272C mAb-peptide 2	2129	nd
E272C mAb-peptide 3	1514	nd
E272C mAb-peptide 4	1631	nd
E272C mAb-peptide 5	1118	1946
E272C mAb-peptide 6	1035	1611

Example 4.3: Functional activity in a GLP-1 cAMP assay

Before assay set up, assay buffer was prepared as 1x stimulation buffer (Cisbio #62AM4PEJ) containing 500uM IBMX (Sigma, #I5879). Compounds were 4-fold serially diluted 10-points in assay buffer using the Bravo V11 Machine (Agilent) and 5µL of compounds were transferred to the OptiPlate-384 (PerkinElmer, #6007290) to reach as highest concentration 20 nM in the assay plate. The plate was centrifuged for 5 sec at 1000rpm.

HEK293 cells expressing GLP-1R (constructed by WuXi Apptech) were detached using 0.25% Trypsin at 37°C for a couple of minutes. When cells detached, cell suspension was diluted in 10mL of HBSS (Invitrogen, #14025) and transferred into a 15mL tube. Cells were centrifuged at 1000rpm for 5minutes at room temperature. Supernatant was removed and cell pellet resuspended in 10mL HBSS. Cells were re-suspended in assay buffer to a final concentration of 0.2Mio cells/mL. 10µL of this cell solution was transferred to the Optiplate-384.

The plate was incubated for 30min at 23°C before adding 10µL cAMP working detection solution (38 parts of cAMP Lysis Buffer, 1 part of cAMP-D2, 1 part anti-cAMP cryptate reagent) as described by the provider (CisBio, #62AM4PEJ). The plate was covered with TopSeal-A film and incubated for 60min at room temperature. TopSeal-A film was removed, and signal analyzed using the EnVision2015 (PerkinElmer).

Tested in this assay were Peptides 1-6 as well as the corresponding E272C conjugates thereof. Results are shown in the following Table 8 and Figure 4. It could be demonstrated that the conjugates remain highly active and fully functional in the cAMP assay.

Table 8:

Sample ID	EC50 (pM)	% Activation @ Max dose	Sample ID	EC50 (pM)	% Activation @ Max dose
Peptide 1	10	68	Peptide 2	9	95
Peptide 3	8	100	Peptide 4	11	110
Peptide 5	7	101	Peptide 6	6	121
E272C mAb-peptide 1	214	91	E272C mAb-peptide 2	11	102
E272C mAb-peptide 3	201	101	E272C mAb-peptide 4	36	92
E272C mAb-peptide 5	86	96	E272C mAb-peptide 6	80	97

Example 4.4: Conclusion from in vitro studies

It was surprisingly found that the conjugation of agonistic GLP-1 peptides to ActRII antibodies does not substantially affect the activity of either part of the molecule. Specifically, the conjugated agonistic GLP-1 peptide does not affect binding and activity of the ActRII antibody and fully translates into functional ActRII activity. Likewise, the activity of the agonistic GLP-1 peptide when conjugated to the ActRII antibody is essentially equivalent to the isolated (unconjugated) agonistic GLP-1 peptide. Both parts of the molecules, i.e. the agonistic GLP-1 peptide and the ActRII antibody, remain fully active despite the conjugation of the respective other part of the molecule.

This finding provides great advantages for the development of a respective therapeutic agent. Compared to a combination therapy consisting of the two individual moieties, a conjugation product only requires a single formulation, a single route of administration, and avoids the necessity to identify a compatible dosage regimen for two individual molecules.

Example 5: In vivo efficacy study

The in vitro studies shown herein above were experimentally verified by respective in vivo studies. Diet-induced obesity (DIO) mice were used as a model to measure weight loss and body composition improvements after treatment with anti-ActRIIA/B-GLP-1 conjugates.

C57BL/6JRj male mice obtained from Janvier Labs were subjected to a 60% high fat diet (HFD) from the age of 5 weeks onwards for a period of 21 weeks before study enrolment. Animals were randomized before dosing into treatment groups based on bodyweight and body composition measurements. The conjugates (or their respective vehicle, 0.9% NaCl) were administered twice-weekly via intraperitoneal (IP) injection for 2 weeks, whilst semaglutide (or its respective vehicle, phosphate buffered saline containing 0.1 % bovine serum albumin), was administered via daily subcutaneous (SC) injections of 10 nmol/kg for 17 days. The treatment groups consisted of vehicle only (IP and SC route), semaglutide SC (+ IP vehicle), E272C mAb-peptide 5 (10 mg/kg), E272C mAb-peptide 6 (2.5 mg/kg or 10mg/kg) + SC vehicle. HFD was continued during treatment. The bodyweight of the animals was recorded daily. Food intake was measured daily during the first treatment week and biweekly thereafter. The body composition (fat and lean mass) was analyzed weekly by magnetic resonance imaging (MRI).

The therapeutic activity of the conjugates of the present disclosure could be confirmed in this in vivo study. Of importance, the conjugates of the present disclosure led to pronounced decrease of fat body mass without loss of lean (muscle) mass, whereas the loss of body weight of semaglutide is attributable to the loss of fat mass and a substantial amount lean (muscle) body mass. Upon treatment with the conjugates lean body mass is fully preserved.

Example 6: Fusion antibodies

In this experiment it was tested, if the agonistic GLP-1 peptides could also be attached to the ActRII antibody by way of a genetic fusion. To do so, the GLP-1 peptides were genetically fused to the N-terminus of the variable heavy chain of the ActRII antibodies.

Two different agonistic GLP-1 peptides were utilized, both of which had three amino acid substitution compared to native GLP-1. The GLP-1 peptides were separated from the variable heavy chain by a short glycine-serine rich linker. The amino acid sequence of the fusion constructs tested are shown in the following table.

Table 9 (underlined: GLP-1 agonist; italics: linker; bold: start of variable heavy chain):

SEQ ID No.	Designation	Amino acid sequence
44	G8 fusion	HGEGTFTSDVSSYLEEQAAKEFIAWLVK*GGGGGGGSGGGGSGGGGS***QVQ**
45	S8 fusion	HSEGTFTSDVSSYLEEQAAKEFIAWLVK*GGGGGGGSGGGGSGGGGS***QVQ**
46	G8 peptide	HGEGTFTSDVSSYLEEQAAKEFIAWLVK
47	S8 peptide	HSEGTFTSDVSSYLEEQAAKEFIAWLVK
48	linker	GGGGGGGSGGGGSGGGGS

In some fusion constructs GLP-1 was fused to the N-terminus of both variable heavy chains (double fusions), whereas in other constructs GLP-1 was fused to the N-terminus of only one of the variable heavy chains (single fusions). Unfused control antibodies and semaglutide were used as controls. The constructs tested are depicted in Figure 6. Assays were performed as described in Example 4 herein above.

EC50's as determined in an ActRIIA/B dependent SMAD activation RGA assay using activin A are shown in the following table.

Table 10:

Construct	EC50 [pM]
Unfused control	456
G8 double fusion	1889
S8 double fusion	1349
G8 single fusion	694
S8 single fusion	509

In this assay the single fusion constructs showed a comparable efficiency to the unfused control molecule, whereas the double fusions showed a slightly higher EC50.

EC50's as determined in an hGLP-1R dependent cAMP/PKA activation RGA assay are shown in the following table.

Table 11:

Construct	EC50 [pM]
Semaglutide	122.4
G8 double fusion	21.8

S8 double fusion	17.1
G8 single fusion	61.7
S8 single fusion	107.6

In this assay the single and the double fusion constructs where at least as potent as semaglutide, with the double fusion constructs showing a higher efficiency than the single fusion constructs.

Claims

1. An immunoconjugate comprising an ActRII receptor antibody and an agonistic GLP-1 peptide.

2. The immunoconjugate according to claim1, wherein said ActRII receptor antibody is specific for a polypeptide of SEQ ID No. 12.

3. The immunoconjugate according to claim 1 or 2, wherein said ActRII receptor antibody is cross-reactive with a polypeptide of SEQ ID No. 11.

4. The immunoconjugate according to any one of the preceding claims, wherein said ActRII receptor antibody is binds to the same epitope as an antibody comprising a variable heavy chain comprising the CDR amino acid sequences of SEQ ID NO: 1 (CDRH1), SEQ ID NO: 2 (CDRH2), and SEQ ID NO: 3 (CDRH3); and comprise a variable light chain comprising the CDR amino acid sequences of SEQ ID NO: 4 (CDRL1), SEQ ID NO: 5 (CDRL2), and SEQ ID NO: 6 (CDRL3).

5. The immunoconjugate according to any one of the preceding claims, wherein said agonistic GLP-1 peptide is selected from

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-K(MiniPEGAcBr) (SEQ ID No. 14),

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-KKgGluC16(MiniPEGAcBr) (SEQ ID No. 15),

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG-K(GGGGS)3AcBr (SEQ ID No. 16),

HAibEGTFTSDVSSYLEGQAA-K(MiniPEGAcBr)-EFIAWLVRGRG (SEQ ID No. 17),

HAibEGTFTSDVSSYLEGQAA-KKgGluC16(MiniPEGAcBr)-EFIAWLVRGRG (SEQ ID No. 18),

HAibEGTFTSDVSSYLEGQAA-K(GGGGS)3AcBr-EFIAWLVRGRG (SEQ ID No. 19),

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-K(MiniPEGAcBr) (SEQ ID No. 20),

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-KKgGluC16(MiniPEGAcBr) (SEQ ID No. 21), and

HAibEGTFTSDVSSYLEGQAAKEFIAWLVRGRG-K(GGGGS)3AcBr (SEQ ID No. 22).

6. The immunoconjugate according to any one of claims 1-4, wherein said agonistic GLP-1 peptide is GLP-1 dual agonist or a GLP-1 triagonist.

7. The immunoconjugate according to claim 6, wherein said agonistic GLP-1 peptide is selected from

YAibEGTFTSDYSIYLDKQAAAibEFVNWLLAGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 23),

YAibEGTFTSDYSIYLDKQAAAibEFV-K(MiniPEGAcBr)-WLLAGGPSSGAPPPSK-NH2 (SEQ ID No. 24),

YAibEGTFTSDYSIYLDKQAAAibEFVNWLLAG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 25),

YAibEGTFTSDYSIYLDKQAAAibEFV-K(MiniPEGAcBr)-WLLAG-NH2 (SEQ ID No. 26),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 27),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 28),

YAibAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 29),

YAibQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLbAlaG-K(MiniPEGAcBr)-NH2 (SEQ ID No. 30),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSarGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 31),

Ac-hAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSar-K(MiniPEGAcBr)-NH2 (SEQ ID No. 32),

YAibAQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSarGPSSGAPPPSK(MiniPEGAcBr)-NH2 (SEQ ID No. 33),

YAibQGTFTDSK(gGluC16)SKYLDERAAQDFVQWLLisoAspSar-K(MiniPEGAcBr)-NH2 (SEQ ID No. 34),

Ac-hAQGTFTSD-K(MiniPEGAcBr)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-NH2 (SEQ ID No. 35),

Ac-hAQGTFTSDKSKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 36),

Ac-hAQGTFTSD-K(MiniPEGAcBr)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(gGluC16)-NH2 (SEQ ID No. 37), and

Ac-hAQGTFTSD-K(gGluC16)-SKYLDERAAQDFVQWLLEGGPSSGAPPPS-K(MiniPEGAcBr)-NH2 (SEQ ID No. 38).

8. The immunoconjugate according to any one of claims 2-6, wherein said agonistic GLP-1 peptide is conjugated to said ActRII receptor antibody via cysteine engineering.

9. The immunoconjugate according to claim 7, wherein said agonistic GLP-1 peptide is conjugated to said ActRII receptor antibody at position 272, 361, 384, 389 or 434 of the ActRII receptor antibody.

10. The immunoconjugate according to any one of the preceding claims, wherein said ActRII receptor antibody has a silencing mutation.

11. A pharmaceutical composition comprising an immune conjugate according to any one of the preceding claims.

12. An immunoconjugate according to any one of claims 1-10 or a pharmaceutical composition according to claim 11 for use in medicine.

13. An immunoconjugate or a pharmaceutical composition for use according to claim 12, wherein said use in medicine is the use in the treatment of a metabolic disorder.

14. An immunoconjugate or a pharmaceutical composition for use according to claim 13, wherein said metabolic disorder is selected from the group consisting of: obesity, diabetes, metabolic syndrome, anti-psychotic drug-associated obesity, glucocorticoid-induced obesity, hypothalamic obesity associated with craniopharyngioma, Prader-Willi syndrome, and a monogenetic disorder associated with obesity.

15. An immunoconjugate or a pharmaceutical composition for use according to claim 14, wherein said treatment is useful for an obesity related co-morbidity, wherein the condition is selected from the group of: glucose intolerance, prediabetes, insulin resistance, high triglycerides, overweight associated physical impairment, osteoporosis, renal disease, obstructive sleep apnea, sexual

hormones impairment, endocrine reproductive disorders, osteoarthritis, gastrointestinal cancers, dyslipidemia, hypertension, heart failure, coronary heart disease, stroke, and/or gallstones.

16. An ActRII receptor antibody, wherein said ActRII receptor antibody is fused to an agonistic GLP-1 peptide.