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[0006] Amino acid substitutions that reduce binding of the Fc region to Fc γ R may result in a reduced thermal stability of the Fc region (see, for example, International Publication No. WO 2020/086776 and J Biol Chem; 292(5):1865-75 (2017)).

[0007] International Publication No. WO 2013/118858 describes amino acid substitutions in the Fc region that improve thermal stability as well as increase or decrease an effector function. WO 2013/118858 shows that a combination of amino acid substitutions, each of which independently improves thermal stability, does not necessarily improve thermal stability in an additive manner.

SUMMARY

[0008] Described herein are Fc variants with high thermal stability and attenuated effector function. One aspect of the disclosure relates to a polypeptide comprising one or more variant Fc regions, each of the variant Fc regions comprising two CH2 domains, wherein at least one CH2 domain in at least one of the variant Fc regions is a variant CH2 domain comprising amino acid substitutions at positions 234, 235 and 265 (all positions expressed by EU numbering), wherein the amino acid residues at positions 234, 235, and 265 of the variant CH2 domain are Ala, Ala, and Gly, respectively, or Ala, Ala, and Asn, respectively, and wherein each of the one or more variant Fc regions is a variant IgG1 Fc region, a variant IgG4 Fc region or a variant IgG1/IgG4 Fc region.

[0008a] In another aspect, provided herein is a polypeptide comprising one or more variant Fc regions, each of the variant Fc regions comprising two CH2 domains, wherein at least one CH2 domain in at least one of the variant Fc regions is a variant CH2 domain comprising amino acid substitutions at positions 234, 235 and 265 (all positions expressed by EU numbering), wherein the amino acid residues at positions 234, 235, and 265 of the variant CH2 domain are Ala, Ala,

and Gly, respectively, or Ala, Ala, and Asn, respectively, wherein each of the one or more variant Fc regions is a variant IgG1 Fc region, a variant IgG4 Fc region or a variant IgG1/IgG4 Fc region, and wherein the polypeptide has reduced binding affinity to an Fc γ RI, an Fc γ RII and an Fc γ RIII as compared to a parent polypeptide comprising a parent CH2 domain without amino acid substitutions at positions 234, 235 and 265.

[0008b] In another aspect, provided herein is a fusion protein comprising the polypeptide described herein and a heterologous polypeptide

[0008c] In another aspect, provided herein is a polypeptide-drug conjugate comprising the polypeptide described herein conjugated to one or more drugs or modifying agents.

[0008d] In another aspect, provided herein is a nucleic acid comprising a nucleotide sequence encoding the polypeptide described herein or a portion thereof.

[0008e] In another aspect, provided herein is a host cell comprising the nucleic acid described herein.

[0008f] In another aspect, provided herein is a method for producing the polypeptide described herein or a portion thereof, the method comprising culturing the host cell described herein in a medium under conditions suitable for expression of the polypeptide or the portion thereof, and recovering the polypeptide or the portion thereof from the medium or the host cell.

[0008g] In another aspect, provided herein is a pharmaceutical composition comprising the polypeptide described herein, the fusion protein described herein, or the polypeptide-drug conjugate described herein, and a pharmaceutically acceptable carrier.

[0008h] In another aspect, provided herein is a polypeptide described herein, a fusion protein described herein, or a polypeptide-drug conjugate described herein, for use in therapy.

[0008i] In another aspect, provided herein is the use of the polypeptide described herein, the fusion protein described herein or polypeptide-drug conjugate described herein in the manufacture of a medicament for treatment of a cancer, an immune disease, an inflammatory disease or an infectious disease.

[0009] Another aspect of the disclosure relates to a molecule comprising a polypeptide as described herein.

[0010] Another aspect of the disclosure relates to a polypeptide-drug conjugate comprising a polypeptide as described herein conjugated to one or more drugs or modifying agents.

L234A/L235A/D265N (LALA-DN) mutations into the Fc region of IgG1 WT; a variant obtained by introducing L234A/L235A/D265Q (LALA-DQ) mutations into the Fc region of IgG1 WT; and a variant obtained by introducing L234A/L235A/D265T (LALA-DT) mutations into the Fc region of IgG1 WT, as evaluated by an SPR method.

[0028] **Figure 6** shows binding activity for the antibodies shown in Figure 5 to cynomolgus Fc γ RI, as evaluated by an SPR method.

[0029] **Figure 7A** shows a change in temperature-dependent heat capacity of the LALA-DG variant of the anti-EGFR antibody, panitumumab, as measured by DSC. The number near each peak represents the T_m .

[0030] **Figure 7B** shows a change in temperature-dependent heat capacity of the LALA-DN variant of the anti-EGFR antibody, panitumumab, as measured by DSC. The number near each peak represents the T_m .

[0031] **Figure 7C** shows a change in temperature-dependent heat capacity of the LALA-DQ variant of the anti-EGFR antibody, panitumumab, as measured by DSC. The number near each peak represents the T_m .

[0032] **Figure 7D** shows a change in temperature-dependent heat capacity of the LALA-DT variant of the anti-EGFR antibody, panitumumab, as measured by DSC. The number near each peak represents the T_m .

[0033] **Figure 8** shows time-dependent changes in blood concentrations of IgG1 WT, LALA variant, and LALA-DG variant of the anti-EGFR antibody, panitumumab, in mice after intravenous (i.v.) administration. Error bar in the figure indicates the standard deviation ($n = 3$).

[0034] **Figure 9A** shows binding activity to human Fc γ RI for the LALA-DG variant of the anti-EGFR antibody, panitumumab, and a variant obtained by introducing L234A/L235A/D265G/P329A (LALA-DGPA) mutations into the Fc region of IgG1 WT, as evaluated by an SPR method.

[0035] **Figure 9B** shows binding activity for the antibodies shown in Figure 9A to cynomolgus Fc γ RI, as evaluated by an SPR method.

[00152] [20] A pharmaceutical composition comprising the polypeptide according to any one of [1] to [14], the molecule according to [15] or [16], or the polypeptide-drug conjugate according to [17], and a pharmaceutically acceptable carrier.

[00153] [21] A polypeptide according to any one of [1] to [14], the molecule according to [15] or [16], or polypeptide-drug conjugate according to [17], for use in therapy.

[00154] [22] The polypeptide, molecule or polypeptide-drug conjugate for use according to [21], wherein the therapy comprises treatment of a cancer, an immune disease, an inflammatory disease or an infectious disease.

EXAMPLES

[00155] The following Examples are provided for illustrative purposes and are not intended to limit the scope of the invention in any way.

COMPARATIVE EXAMPLE 1: Evaluation of Binding Activity of an Anti-EGFR Antibody with Known Effectorless Mutations to Human FcγRI.

[00156] The binding activity of an anti-EGFR antibody, into which known effectorless mutations were introduced, to human FcγRI (hFcγRI), was evaluated using Biacore™ T200 (Cytiva, Marlborough, MA). The Fc region of anti-EGFR antibody Panitumumab, which is originally a human IgG2 antibody, was replaced by that of human IgG1 and the resulting antibody is termed IgG1 WT. The amino acid sequences of the light chain and the heavy chain of IgG1 WT are represented by SEQ ID Nos: 13 and 14, respectively (see Figures 16A and 16B). IgG1 WT and the following variants were evaluated: a variant obtained by incorporating L234A/L235A (LALA) mutations into the Fc region of IgG1 WT; a variant obtained by introducing L234A/L235A/D265A (LALA-DA) mutations into the Fc region of IgG1 WT; and a variant obtained by introducing L234A/L235A/P329G (LALA-PG, described in International Publication No. WO 2012/130831) mutations into the Fc region of IgG1 WT. The noted mutations were introduced into both CH2 domains of the Fc region. These anti-EGFR antibodies (also referred as “variants of the anti-EGFR