Antigen Binding Constructs

Background

The Vascular Endothelial Growth Factor (VEGF) family of growth factors and their receptors are essential regulators of angiogenesis and vascular permeability. The VEGF family comprises VEGF-A, PIGF (placenta growth factor), VEGF-B, VEGF-C, VEGF-E and snake venom VEGF and each is thought to have a distinct role in vascular patterning and vessel development. Due to alternative splicing of mRNA transcribed from a single 8-exon gene, VEGF–A has at least 9 subtypes (isoforms) identified by the number of amino acids remaining after signal peptide cleavage. For example, in humans the most prominent isoform is $VEGF_{165}$, which exists in equilibrium between a soluble and cell associated form. Longer isoforms ($VEGF_{183}$, $VEGF_{189}$ & $VEGF_{206}$) possess C-terminal regions that are highly positively charged and mediate association with cell surface glycans and heparin that modulates their bioavailability. All VEGF-A isoforms form homodimers with the association occurring via a core of approximately 110 N-terminal residues that constitutes the receptor-binding VEGF fragment. Under normal circumstances, and in the centre of solid tumours, expression of VEGF is principally mediated by hypoxic conditions, signifying a shortage of vascular supply. The hypoxia causes dimerization of the hypoxia inducible factor HIF-1α with the constitutively expressed HIF-1α, forming a transcription factor that binds to hypoxic response elements in the promoter region of the VEGF gene. Under normoxia, the HIF-1α protein undergoes ubiquitin-mediated degradation as a consequence of multiple proline hydroxylation events. Other tumour-associated VEGF up-regulation occurs due to activation via oncogene pathways (i.e. ras) via inflammatory cytokines & growth factors as well as by mechanical forces.

The active VEGF homodimer is bound at the cell surface by receptors of the VEGFR family. The principal vascular endothelium associated receptors for VEGF-A are VEGFR1 (Flt1) and VEGFR2 (Flk-2; KDR). Both receptors are members of the tyrosine kinase family and require ligand-mediated dimerization for activation. Upon dimerization the kinase domains undergo autophosphorylation, although the extent of the kinase activity in VEGFR2 is greater than that in VEGFR1. It has been demonstrated that the angiogenic signalling of VEGF is mediated largely through VEGFR2, although the affinity of VEGF is approximately 3-fold greater for VEGFR1 (KD ~30pM compared with 100pM for VEGFR2). This has led to the proposal that VEGFR1 principally acts as a decoy receptor to sequester VEGF and moderate the extent of VEGFR2 activation. Although VEGFR1 expression is associated with some tumours, its principal role appears to be during embryonic development & organogenesis. VEGF-A_{165} is also bound by the neuropilin receptors NRP1 & NRP2. Although these receptors lack TK domains, they are believed to acts as co-receptors for VEGFR2 and augment signalling by transferring the VEGF to the VEGFR2.

Numerous studies have helped confirm VEGF-A as a key factor in tumour angiogenesis. For example VEGF-A is expressed in most tumours and in tumour associated stroma. In the absence of a well developed and expanding vasculature system to support growth, tumour cells become necrotic and apoptotic thereby imposing a limit to the increase in tumour volume (of the order 1 mm3) that can result from continuous cell proliferation. The expression of VEGF-A is highest in hypoxic tumour cells adjacent to necrotic areas indicating that the induction of VEGF-A by hypoxia in growing tumours can change the balance of activators and inhibitors of angiogenesis, leading to the growth of new blood vessels in the tumour. Consistent with this hypothesis, a number of approaches, including small-molecular weight tyrosine kinase inhibitors, monoclonal antibodies, antisense oligonucleotides etc., that inhibit or capture either VEGF-A or block its signalling receptor, VEGFR-2, have been developed as therapeutic agents.

VEGF-A has also been implicated in a number of ocular diseases, such as age-related macular degeneration (AMD), wet AMD, geographic atrophy, diabetic retinopathy, retinal vein-occlusive diseases, diabetic macular oedema and corneal vascularisation. VEGF-A is produced by various ocular cell types in response to hypoxia and has a number of functions, including promoting vascular permeability and stimulating endothelial cell growth.

AMD is defined as an abnormality of the retinal pigment epithelium, which leads to degeneration of the overlying photoreceptor in the macula and results in loss of central vision. AMD represents a major public health burden and it is estimated that over 9 million people in the US have intermediate or advanced forms of AMD. Early AMD is characterised by drusen and hyper or hypopigmentation of the retinal pigment epithelium without loss of vision. Advanced AMD, where loss of vision occurs, can present as geographic atrophy or choriodal neovascularisation (CNV). CNV, which is also referred to as wet AMD, is a result of the abnormal growth of blood vessels.

Ranibizumab (Lucentis), bevacizumab (Avastin) and aflibercept (Eylea) are examples of anti-VEGF therapies, which are commonly administered for neovascular AMD. Despite the presence of such therapies, there exists a need for further therapies for the treatment of AMD and other ocular diseases.

Summary of the Invention

The present invention is directed to antigen binding constructs comprising one or two epitope binding domains separated by a single chain Fc region of an antibody, wherein each epitope binding domain is capable of binding to VEGF.

In one aspect, each epitope binding domain of an antigen binding construct of the present invention is a domain antibody, also referred to as a dAb.

In a further aspect, the present invention is directed to the following antigen binding construct:

a) DOM 15-26-597 – AS– Fc (SEQ ID No. 110) – Fibronectin x 4 – DT02-K-044-085; or

b) DOM 15-26-597 – IgG1 hinge – Fc (SEQ ID No. 110) – Fibronectin x 4– DT02-K-044-085; or

c) A variant of (a) or (b) having at least 97% amino acid identity across the whole sequence; or

d) A variant of (a) or (b), wherein each dAb sequence has at least 97% sequence identity to the sequence of the specific dAb.

The present invention is further directed to dimers comprising two antigen binding constructs of the invention, pharmaceutical compositions comprising said dimers and their use in the treatment of diseases associated with VEGF signalling, such as diabetic macular edema (DME), wet age-related macular degeneration (Wet AMD), diabetic retinopathy, retinal vein occlusion (RVO), and corneal neovascularisation, and polynucleotide sequences encoding said antigen binding constructs.

Figures

Figure 1: ELISA, with EC50 values, showing comparison of DMS1529, DMS1576 and Bevacizumab (Avastin) binding to human $VEGF_{165}$.

Figure 2A: Binding of Vh dAb-Fc DMS1576 to VEGF after pre-saturation with Vk Fc-dAb DMS30000 3ug/mL by MSD.

Figure 2B: Binding of Vk Fc-dAb DMS30000 to VEGF after pre-saturation with Vh dAb-Fc DMS1576 3ug/mL by MSD .

Figure 3: Weighted analysis of variance on maximum inhibition of VEGF induced Human Umbilical Cord Endothelial Cell (HUVEC) proliferation assay from anti-VEGF Vh dAb-Fc, Vk Fc-dAb and dAb-Fc-dAb molecules compared to Bevacizumab (Avastin).

Figure 4: Binding of DMS1529 and DMS30000 to mouse $VEGF_{164}$ by MSD.

Detailed Description of the Invention

The present invention is directed to antigen binding constructs comprising two epitope binding domains separated by a single chain Fc region of an antibody, wherein each epitope binding domain in capable of binding to VEGF.

In one aspect, each epitope binding domain of an antigen binding construct of the present invention is a domain antibody, also referred to as a dAb.

As used herein, the term "epitope binding domain" refers to a domain that specifically binds an antigen or epitope independently of a different variable region or domain. This may be a domain antibody (dAb) or it may be a domain which is a derivative of a scaffold selected from the group consisting of CTLA-4 (Evibody); lipocalin; Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); Heat shock proteins such as GroEl and GroES; transferrin (trans-body); ankyrin repeat protein (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human γ-crystallin and human ubiquitin (affilins); PDZ domains; scorpion toxin (charybdotoxin); kunitz type domains of human protease inhibitors; PDZ-domains of the Ras-binding protein AF-6; and fibronectin (adnectin); which has been subjected to protein engineering in order to obtain binding to a ligand other than the natural ligand.

As used herein, the term "domain" is a folded protein structure which has tertiary structure independent of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain.

As used herein, the term "domain antibody" refers to a folded polypeptide domain comprising sequences characteristic of antibody variable domains. It therefore includes complete antibody variable domains such as VH, VHH and VL and modified antibody variable domains, for example, in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains, or antibody variable domains which have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains which retain at least the binding activity and specificity of the full-length domain. A single variable domain is capable of binding an antigen or epitope independently of a different variable region or domain. A "domain antibody" or "dAb$^{(TM)}$" may also be referred to as a "single variable domain". A domain antibody may be a human domain antibody, but also includes single domain antibodies from other species such as rodent (for example, as disclosed in WO 00/29004), nurse shark and Camelid VHH dAbs. Camelid VHH are immunoglobulin single variable domain polypeptides that are derived from species including camel, llama, alpaca, dromedary, and guanaco, which produce heavy chain antibodies naturally devoid of light chains. Such VHH domains may be humanised according to standard techniques available in the art, and such domains are considered to be "domain antibodies". As used herein VH includes camelid VHH domains. NARV are another type of immunoglobulin domain antibody, which were identified in cartilaginous fish including the nurse shark (Mol. Immunol. 44, 656-665 (2006). These domains are also known as Novel Antigen Receptor variable region (commonly abbreviated to V(NAR) or NARV).

As used herein, the term "single chain Fc region of an antibody" refers to a single heavy chain Fc region of an IgG, such as an IgG1, IgG2, IgG3, iGG4 or IgG4PE, or an IgA antibody. A single heavy chain Fc region may comprise one or more of the CH1, CH2 and CH3 constant region antibody domains, for example all three constant region antibody domains or just the CH2 and CH3 domains. In addition to comprising one or more of

the CH1, CH2 and CH3 constant region antibody domains, the single heavy chain FC region of an antibody may further comprise a hinge region of an antibody (such a region normally found between the CH1 and CH2 domains).

In one aspect, the single chain Fc region of an antibody is a single IgG1 heavy chain, for example a single IgG1 heavy chain comprising the CH2 and CH3 antibody constant domains.

In a further aspect, the single chain Fc region of an antibody is the IgG1 sequence of SEQ ID No. 110.

In a further aspect, the single chain Fc region of an antibody is the following sequence:
QASSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

In one aspect the N terminus of the Fc sequence starts “AS” or no N-terminal amino acid at start of Fc (Fc sequence starts ASTHTCPPC or THTCPPC).

In another aspect the Fc region comprises a mutation within the N-terminus of the Fc, for example THTCPPC is replaced by TATCPPC. For example THTCPPC is replaced by THPCPPC (eg SEQ ID 83 and 84). Such mutations may be present in any sequence disclosed herein, and are variant sequences of those specific sequences disclosed herein.

The Fc region of an antibody may be selected for its degree of effector function.

The term “Effector Function” as used herein is meant to refer to one or more of Antibody Dependant Cell-mediated Cytotoxic (ADCC) , Complement–Dependant Cytotoxic (CDC) mediated responses, Fc-mediated phagocytosis and antibody recycling via the FcRn receptor. For IgG antibodies, effector functionalities including ADCC and ADCP are mediated by the interaction of the heavy chain constant region with a family of Fcγ receptors present on the surface of immune cells. In humans these include FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16). Interaction between the antigen binding protein bound to antigen and the formation of the Fc/ Fcγ complex induces a range of effects including cytotoxicity, immune cell activation, phagocytosis and release of inflammatory cytokines.

The interaction between the constant region of an antigen binding protein and various Fc receptors (FcR) is believed to mediate the effector functions of the antigen binding protein. Significant biological effects can be a consequence of effector functionality, in particular, antibody-dependent cellular cytotoxicity (ADCC), fixation of complement (complement dependent cytotoxicity or CDC), and half-life/clearance of the antigen

binding protein. Usually, the ability to mediate effector function requires binding of the antigen binding protein to an antigen and not all antigen binding proteins will mediate every effector function.

Effector function can be measured in a number of ways including for example via binding of the FcγRIII to Natural Killer cells or via FcγRI to monocytes/macrophages to measure for ADCC effector function. For example an antigen binding protein of the present invention can be assessed for ADCC effector function in a Natural Killer cell assay. Examples of such assays can be found in Shields et al, 2001 The Journal of Biological Chemistry, Vol. 276, p6591-6604; Chappel et al, 1993 The Journal of Biological Chemistry, Vol 268, p25124-25131; Lazar et al, 2006 PNAS USA, 103; 4005-4010.Examples of assays to determine CDC function include that described in 1995 J Imm Meth 184:29-38.

Some isotypes of human constant regions, in particular IgG4 and IgG2 isotypes, essentially lack the functions of (a) activation of complement by the classical pathway; and (b) antibody-dependent cellular cytotoxicity. Various modifications to the heavy chain constant region of antigen binding proteins may be carried out depending on the desired effector property. IgG1 constant regions containing specific mutations have separately been described to reduce binding to Fc receptors and therefore reduce ADCC and CDC (Duncan et al. Nature 1988, 332; 563-564; Lund et al. J. Immunol. 1991, 147; 2657-2662; Chappel et al. PNAS USA 1991, 88; 9036-9040; Burton and Woof, Adv. Immunol. 1992, 51;1-84; Morgan et al., Immunology 1995, 86; 319-324; Hezareh et al., J. Virol. 2001, 75 (24); 12161-12168).

Human IgG1 constant regions containing specific mutations or altered glycosylation on residue Asn297 have also been described to enhance binding to Fc receptors. In some cases these mutations have also been shown to enhance ADCC and CDC (Lazar et al. PNAS USA 2006, 103; 4005-4010; Shields et al. J Biol Chem 2001, 276; 6591-6604; Nechansky et al. Mol Immunol, 2007, 44; 1815-1817).

In one embodiment of the present invention, such mutations are in one or more of positions selected from 239, 332 and 330 (IgG1), or the equivalent positions in other IgG isotypes. Examples of suitable mutations are S239D and I332E and A330L. In one embodiment the antigen binding protein of the invention herein described is mutated at positions 239 and 332, for example S239D and I332E or in a further embodiment it is mutated at three or more positions selected from 239 and 332 and 330, for example S239D and I332E and A330L (EU index numbering).

In an alternative embodiment of the present invention, there is provided an antigen binding protein comprising a heavy chain constant region with an altered glycosylation profile such that the antigen binding protein has enhanced effector function. For example, wherein the antigen binding protein has enhanced ADCC or enhanced CDC or wherein it has both enhanced ADCC and CDC effector function. Examples of suitable methodologies to produce antigen binding proteins with an altered glycosylation profile are

described in WO2003011878, WO2006014679 and EP1229125, all of which can be applied to the antigen binding proteins of the present invention.

The antigen binding constructs of the present invention comprise epitope binding domains that are capable of binding to VEGF. As used herein, the term "VEGF" is a reference to any VEGF molecule, in particular VEGF-A, for example human VEGF-A, and including any isoform of VEGF-A, such as $VEGF_{165}$.

Antigen binding constructs of the present invention, in one aspect, comprise two epitope binding domains separated by a single chain Fc region of an antibody. By separated it is meant that the epitope binding domains are not directly attached to one another. In one aspect the epitope binding domains are located at opposite ends of the Fc region. One epitope binding domain is attached to the N-terminus and the other it attached to the C-terminus. Each epitope binding domain is independently selected and such domains may bind the same epitope on VEGF or different epitopes.

In one aspect, the epitope binding domain attached to the N-terminus end of the Fc region of an antibody, in an antigen binding construct of the present invention, is a heavy or light chain dAb, wherein the light chain dAb may be a kappa or lambda light chain.

In a further aspect, where antigen binding constructs of the present invention have an epitope binding domain attached to the C-terminus, the epitope binding domain is an light chain dAb, wherein the light chain dAb may be a kappa or lambda light chain.

In a yet further aspect, where antigen binding constructs of the present invention comprise two epitope binding domains, the one attached to the N-terminus of the Fc region is a heavy chain dAb and the one attached to the C-terminus of the Fc region is a light chain dAb.

In a yet further aspect, where antigen binding constructs of the present invention comprise two epitope binding domains, the one attached to the N-terminus of the Fc region is a light chain dAb and the one attached to the C-terminus of the Fc region is a light chain dAb.

Antigen binding constructs of the present invention may be expressed as a fusion protein or the epitope binding domain may be expressed separately and connected by another means, such as chemical conjugation using methods well known in the art.

Epitope binding domains can be attached directly to the Fc region of an antibody or indirectly through a linker. In constructs where the N-terminus of a dAb is fused to the C-terminus of a Fc region of an antibody, a peptide linker may enhance antigen binding of the dAb. Indeed, the N-terminal end of a dAb is located

closely to the complementarity-determining regions (CDRs) involved in antigen-binding activity. Thus a peptide linker may act as a spacer between the epitope-binding, and the constant domain of the protein scaffold, which may allow the dAb CDRs to more easily reach the antigen, and in some circumstances bind with higher affinity. Furthermore, certain peptide linkers, for examples those greater than 7 amino acids in length, may promote and enable the association of a heavy chain dAb attached to the N-terminus of the Fc region of an antibody to a light chain dAb attached to the C-terminus of the Fc region of an antibody, in heterodimers and homodimers as described herein. Such association may enhance antigen binding and/or other properties of the antigen binding constructs and dimers of the present invention.

When fused at the C-terminal end of the Fc region of the antibody, each dAb may be located in the vicinity of the CH3 domains of the Fc portion. This is not expected to impact on the Fc binding properties to Fc receptors (e.g. FcγRI, II, III and FcRn) as these receptors engage with the C_{H2} domains (for the FcγRI, II and III class of receptors) or with the hinge between the CH2 and CH3 domains (e.g. FcRn receptor). Another feature of such antigen-binding constructs is that both dAbs are expected to be spatially close to each other and provided that flexibility is provided by provision of appropriate linkers, these dAbs may even form homodimeric species, hence propagating the 'zipped' quaternary structure of the Fc portion, which may enhance stability of the construct.

Examples of suitable linkers include amino acid sequences which may be from 1 amino acid to 150 amino acids in length, or from 1 amino acid to 140 amino acids, for example, from 1 amino acid to 130 amino acids, or from 1 to 120 amino acids, or from 1 to 80 amino acids, or from 1 to 50 amino acids, or from 1 to 20 amino acids, or from 1 to 10 amino acids, or from 5 to 18 amino acids, or greater than 7 but less than or equal to 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140 or 150 amino acids. Such sequences may have their own tertiary structure, for example, a linker of the present invention may comprise a single variable domain. The size of a linker in one embodiment is equivalent to a single variable domain. Suitable linkers may be of a size from 1 to 100 Angstroms, for example may be of a size from 20 to 80 angstroms or for example may be of a size from 20 to 60 angstroms or for example less than 40 angstroms, or less than 20 angstroms, or less than 5 angstroms in length.

In one aspect, the linker is greater than 7 and less than or equal to 150 amino acids in length.

Examples of linkers include, but are not limited to, those outlined as SEQ ID No. 57 to 63 and 66 to 82
In one aspect, the linkers are selected from SEQ ID No.'s 58, 60, 62, 63 and 75.

Where an antigen binding construct of the present invention comprises two epitope binding domains, the epitope binding domains may be attached to the Fc region of an antibody by identical or different linkers.

In one aspect, where a linker is used to attach an epitope binding domain to the N-terminus of the Fc region of an antibody, the linker is selected from the group consisting of SEQ ID No. 58, SEQ ID No. 60 and 62. In a further aspect, the N-terminus linker is SEQ ID no. 58 or SEQ ID No. 60.

In one aspect, where a linker is used to attach an epitope bidning domain to the C-terminus of the Fc region of an antibody, the linker is selected from the group consisting of SEQ ID No. 63 and SEQ ID No. 75. A particular preferred linker is SEQ ID No. 63.

In one aspect, linkers of use in the antigen-binding constructs of the present invention may comprise, either alone or in addition to other linkers, one or more sets of GS residues, for example 'GSTVAAPS' or 'TVAAPSGS' or 'GSTVAAPSGS'.

In one embodiment the epitope binding domain is linked to the Fc region of an antibody by the linker '$(PAS)_n(GS)_m$', '$(GGGGS)_n(GS)_m$', '$(TVAAPS)_n(GS)_m$', '$(GS)_m(TVAAPSGS)_n$', '$(PAVPPP)_n(GS)_m$', '$(TVSDVP)_n(GS)_m$', '$(TGLDSP)_n(GS)_m$', wherein n = 1-10, and m = 0-4.

Examples of such linkers include $(PAS)_n(GS)_m$ wherein n=1 and m=1, $(PAS)_n(GS)_m$ wherein n=2 and m=1, $(PAS)_n(GS)_m$ wherein n=3 and m=1, $(PAS)_n(GS)_m$ wherein n=4 and m=1, $(PAS)_n(GS)_m$ wherein n=2 and m=0, $(PAS)_n(GS)_m$ wherein n=3 and m=0, $(PAS)_n(GS)_m$ wherein n=4 and m=0.

Examples of such linkers include $(GGGGS)_n(GS)_m$ wherein n=1 and m=1, $(GGGGS)_n(GS)_m$ wherein n=2 and m=1, $(GGGGS)_n(GS)_m$ wherein n=3 and m=1, $(GGGGS)_n(GS)_m$ wherein n=4 and m=1, $(GGGGS)_n(GS)_m$ wherein n=2 and m=0 (SEQ ID NO:49), $(GGGGS)_n(GS)_m$ wherein n=3 and m=0 (SEQ ID NO:50), $(GGGGS)_n(GS)_m$ wherein n=4 and m=0.

Examples of such linkers include $(TVAAPS)_n(GS)_m$ wherein n=1 and m=1, $(TVAAPS)_n(GS)_m$ wherein n=2 and m=1, $(TVAAPS)_n(GS)_m$ wherein n=3 and m=1 , $(TVAAPS)_n(GS)_m$ wherein n=4 and m=1, $(TVAAPS)_n(GS)_m$ wherein n=2 and m=0, $(TVAAPS)_n(GS)_m$ wherein n=3 and m=0, $(TVAAPS)_n(GS)_m$ wherein n=4 and m=0.

Examples of such linkers include $(GS)_m(TVAAPSGS)_n$ wherein n=1 and m=1 , $(GS)_m(TVAAPSGS)_n$ wherein n=2 and m=1 , $(GS)_m(TVAAPSGS)_n$ wherein n=3 and m=1 , or $(GS)_m(TVAAPSGS)_n$ wherein n=4 and m=1 , $(GS)_m(TVAAPSGS)_n$ wherein n=5 and m=1 , $(GS)_m(TVAAPSGS)_n$ wherein n=6 and m=1 , $(GS)_m(TVAAPSGS)_n$ wherein n=1 and m=0 , $(GS)_m(TVAAPSGS)_n$ wherein n=2 and m=10, $(GS)_m(TVAAPSGS)_n$ wherein n=3 and m=0, or $(GS)_m(TVAAPSGS)_n$ wherein n=0.

Examples of such linkers include $(PAVPPP)_n(GS)_m$ wherein n=1 and m=1 , $(PAVPPP)_n(GS)_m$ wherein n=2 and m=1 , $(PAVPPP)_n(GS)_m$ wherein n=3 and m=1, $(PAVPPP)_n(GS)_m$ wherein n=4 and m=1, $(PAVPPP)_n(GS)_m$ wherein n=2 and m=0, $(PAVPPP)_n(GS)_m$ wherein n=3 and m=0, $(PAVPPP)_n(GS)_m$ wherein n=4 and m=0.

Examples of such linkers include $(TVSDVP)_n(GS)_m$ wherein n=1 and m=1,$(TVSDVP)_n(GS)_m$ wherein n=2 and m=1 , $(TVSDVP)_n(GS)_m$ wherein n=3 and m=1 , $(TVSDVP)_n(GS)_m$ wherein n=4 and m=1, $(TVSDVP)_n(GS)_m$ wherein n=2 and m=0, $(TVSDVP)_n(GS)_m$ wherein n=3 and m=0, $(TVSDVP)_n(GS)_m$ wherein n=4 and m=0.

Examples of such linkers include $(TGLDSP)_n(GS)_m$ wherein n=1 and m=1 , $(TGLDSP)_n(GS)_m$ wherein n=2 and m=1 , $(TGLDSP)_n(GS)_m$ wherein n=3 and m=1 , $(TGLDSP)_n(GS)_m$ wherein n=4 and m=1, $(TGLDSP)_n(GS)_m$ wherein n=2 and m=0, $(TGLDSP)_n(GS)_m$ wherein n=3 and m=0, $(TGLDSP)_n(GS)_m$ wherein n=4 and m=0.

Further linkers that may be used are disclosed in WO 2009068649.

The antigen binding construct may additionally comprise albumin, or a fragment thereof, as a linker, and derived from human serum albumin, such as DETYVPKEFNAETFGS, DETYVPKEFNAETF, EVDETYVPKEFNAETFTFHADGS, EVDETYVPKEFNAETFTFHAD, DDNPNLPRLVRPE, DEMPADLPSLAADF, HKDDNPNLPRLVRPEVDVM, and ENDEMPADLPSLAADFVESKD. The linkers may further comprise some additional residues, for example, they may comprise additional glycine and serine residues or may have amino acids removed from either end of the linker for example in one aspect 5 amino acids are removed. These additional residues may be at the beginning or end of the albumin-derived sequence, or may be within the albumin-derived sequence.

In one aspect, the present invention is directed to antigen binding constructs comprising two epitope binding domains wherein each epitope binding domains is an independently selected dAb and comprises an amino acid sequence which is at least 97% identical to the sequence of DOM 15-26-593, DOM 15-26-597, DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255.

In a further aspect, each epitope binding domain is a dAb independently selected from the group consisting of DOM 15-26-593, DOM 15-26-597, DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 and DT02-K-044-255.

In a further aspect, the dAb attached to the N-terminus of the single chain Fc region comprises an amino acid sequence which is at least 97% identical to the sequence of DOM 15-26-597 or DT02-K-044-085.

In a further aspect, the dAb attached to the N-terminus of the single chain Fc region is DOM 15-26-597 or DT02-K-044-085.

In a further aspect, the dAb attached to the C-terminus of the single chain Fc region comprises an amino acid sequence which is at least 97% identical to the sequence of DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255.

In a further aspect, the dAb attached to the C-terminus of the single chain Fc region is DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255.

In a further aspect, the antigen binding construct of the present invention is:

(a) DOM 15-26-597 – AS– Fc (SEQ ID No. 110) – Fibronectin x 4 – DT02-K-044-085; or

e) (b) DOM 15-26-597 – IgG1 hinge – Fc (SEQ ID No. 110) – Fibronectin x 4– DT02-K-044-085; or

f) (c) A variant of (a) or (b) having at least 97% amino acid identity across the whole sequence; or

g) (d) A variant of (a) or (b), wherein each dAb sequence has at least 97% sequence identity to the sequence of the specific dAb.

The present invention is further directed to antigen binding constructs comprising one epitope binding domain attached to a single chain Fc region of an antibody, wherein the epitope binding domain is a dAb which comprises an amino acid sequence which is at least 97% identical to the sequence of DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255, and further wherein the epitope binding domain is capable of binding to VEGF.

In a yet further aspect, the present invention is directed to antigen binding constructs comprising one epitope binding domain attached to a single chain Fc region of an antibody, wherein the epitope binding domain is a dAb selected from DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255.

In one aspect, the antigen binding construct has the following structure (N to C terminus):

a. Fc – Linker C- DT02-K-044-085 dAb;
b. DOM 15-26-593 dAb –Linker N- Fc- Linker C- DT02-K-044-085 dAb;
c. DOM 15-26-597 dAb – Linker N – Fc – Linker C – DT02-K-044-085 dAb;
d. DT02-K-044-085 dAb-Linker N- Fc- Linker C DT02-K-044-085 dAb;
e. Fc – Linker C- DT02-K-044-251 dAb;
f. DOM15-26-597 dAb –Linker N- Fc- Linker C- DT02-K-044-251 dAb;
g. DT02-K-044-251 dAb –Linker N- Fc- Linker C- DT02-K-044-251 dAb;
h. Wherein for a-g , Linker N can be any of those described in: SEQ ID NO:57-63 & SEQ ID NO:76-82;
i. Wherein for a-g , Linker C can be any of those described in: SEQ ID NO:66-75 & SEQ ID NO:85-94;

j. Wherein for a-g, Fc can be any of those described in: SEQ ID NO:64,65,102 & SEQ ID NO:83,84,110;

k. a variant of any of a-j having at least 97% amino acid identity;

l. a variant of any of a-j wherein the dAb sequence has at least 97% identity to the sequence of the specific dAb;

In a further aspect, the antigen binding construct of the present invention has the following structure:

dAb1-LinkerN-Fc-LinkerC-dAb2

Wherein dAb1 can be defined from:

dAb Sequences	DNA Sequence SEQ ID No.	Amino Acid Sequence SEQ ID No.
.DOM15-26-597	96	104
DT02-K-044-085	97	105
DT02-K-044-251	100	108
DT02-K-044-255	101	109

Wherein LinkerN can be defined from:

N-terminal Linker Sequences	DNA Sequence SEQ ID No.	Amino Acid Sequence SEQ ID No.
AAAS Linker	57	76
AS Linker	58	77
TVAAPS Linker	59	78
Hinge IgG1 Linker	60	79
Hinge IgG3 Linker	61	80
Fibronectin x3 Linker	62	81
Fibronectin x4 Linker	63	82

Wherein Fc can be defined from:

Fc Sequences	DNA Sequence SEQ ID No.	Amino Acid Sequence SEQ ID No.
Fc IgG1	102	110
H2A IgG1 Fc	64	83
T3P IgG1 Fc	65	84

Wherein LinkerC can be defined from:

C-terminal Linker Sequences	DNA Sequence SEQ ID No.	Amino Acid Sequence SEQ ID No.
((GS(TVAAPSGS)x3)) Linker	66	85
Fibronectin x3 Linker	67	86
Fibronectin x4 Linker	68	87
Albumin Domain 1 Linker	69	88
Albumin Domain 2 Linker	70	89
Albumin Domain 3 Linker	71	90
Truncated Albumin Domain3 Linker; Alb Dom 3 -TFHAD	72	91
Gly4Ser 3x Linker	73	92
Gly4Ser 4x Linker	74	93
Helical Linker	75	94

Wherein dAb2 can be defined from:

dAb Sequences	DNA Sequence SEQ ID No.	Amino Acid Sequence SEQ ID No.
DT02-K-044-085	97	105
DT02-K-044-251	100	108
DT02-K-044-255	101	109

Naturally occurring autoantibodies exist in humans that can bind to proteins. Autoantibodies can thus bind to endogenous proteins (present in naïve subjects) as well as to proteins or peptides which are administered to a subject for treatment. Therapeutic protein-binding autoantibodies and antibodies that are newly formed in response to drug treatment are collectively termed anti-drug antibodies (ADAs). Pre-existing antibodies against molecules such as therapeutic proteins and peptides, administered to a subject can affect their efficacy and could result in administration reactions, hypersensitivity, altered clinical response in treated patients and altered bioavailability by sustaining, eliminating or neutralizing the molecule. It could be advantageous to provide molecules for therapy which comprise human immunoglobulin (antibody) single variable domains or dAbs which have reduced immunogenicity (i.e. reduced ability to bind to pre-existing ADAs when administered to a subject, in particular a human subject.

Thus in one aspect of the present invention there is provided a modified dAb or antigen binding construct or dimer comprising such a modified dAb, which has reduced ability to bind to pre-existing antibodies (ADAs) as

compared to the equivalent unmodified molecule. By reduced ability to bind it is meant that the modified molecule binds with a reduced affinity or reduced avidity to a pre-existing ADA. Said modified dAb comprise one or more modifications selected from: (a) a C-terminal addition, extension, deletion or tag, and/or (b) one or more amino acid framework substitutions.

In one aspect the modified dAb or antigen binding construct or dimer comprising such a modified dAb comprises:

a) a C-terminal sequence consisting of the sequence VTVS(S)nX [for a VH dAb] or VEIKpRqX [for a VL dAb]; and also optionally

b) one or more amino acid substitutions at positions 14, 41, 108, 110, or 112 compared to a human germline framework sequence

wherein:

n represents an integer independently selected from 0 or 1;

p and q each represent 0 or 1 such that when p represents 1 q may be 0 or 1 and such that when p represents 0, q also represents 0;

X may be present or absent, and if present represents an amino acid extension of 1 to 8 amino acids residues.

In one aspect, the C-terminal sequence of the dAb is VEIKpRqX, wherein p is 1 and q is 0 and X is absent.

In a further aspect, said modified dAb with reduced binding to pre-existing ADAs has one or more amino acid substitutions wherein said one or more amino acid substitutions are selected from the group consisting of a P14A substitution, a P41A substitution, a L108A substitution, a T110A substitution, a S112A substitution, a P14K substitution, a P14Q substitution, and a P14T substitution.

In a further aspect, X is present, and is an extension of 1 to 8 amino acids, in particular an extension of 1 to 8 amino acids which comprises an alanine residue, for example a single alanine extension, or an AS, AST, ASTK, ASTKG, ASTKGP extension.

In a further aspect, X is present, and is an extension of 1 to 8 amino acids, in particular an extension of 1 to 8 amino acids which comprises an A, AAA or T extension.

In one aspect, the modified dAb can comprise a tag present at the C terminus. The tag can be any tag known in the art for example affinity tags such as myc-tags, FLAG tags, his-tags, chemical modification such as PEG, or protein domains such as the antibody Fc domain

The C terminal addition or extension or tag can be present as a direct fusion or conjugate with the C terminus of the molecule.

Immunoassays well known to those skilled in the art can be used to confirm that the modified dAbs have the desired reduced binding to ADAs.

In a further aspect, the present invention is directed to dimers of the antigen binding constructs disclosed herein. As used herein, the term "dimer" means a polypeptide complex which comprises two antigen binding constructs, i.e two chains that associate with one another to form a dimer. A dimer may be a homodimer, comprising two identical antigen binding constructs of the invention or a heterodimer comprising two different antigen binding constructs of the invention. Homodimers and heterodimers of the present invention may have improved properties, such as affinity, for the target VEGF molecule.

In one aspect, homodimers and heterodimers of the present invention may bind VEGF with a Kd of at least 1mM, for example a Kd of at least 10nM, 1nM, 500pM, 200pM, 100pM, <100pM, 10pM, 5pM, 1pM to antigen as measured by Biacore™, such as the Biacore™ method as described in methods herein.

The in-vivo half life of the antigen binding constructs and dimers of the present invention may be increased by modification of the immunoglobulin constant domain or FcRn (Fc receptor neonate) binding domain.

In adult mammals, FcRn, also known as the neonatal Fc receptor, plays a key role in maintaining serum antibody levels by acting as a protective receptor that binds and salvages antibodies of the IgG isotype from degradation. IgG molecules are endocytosed by endothelial cells, and if they bind to FcRn, are recycled out into circulation. In contrast, IgG molecules that do not bind to FcRn enter the cells and are targeted to the lysosomal pathway where they are degraded.

The neonatal FcRn receptor is believed to be involved in both antibody clearance and the transcytosis across tissues (see Junghans R.P (1997) Immunol.Res 16. 29-57 and Ghetie et al (2000) Annu.Rev.Immunol. 18, 739-766). Human IgG1 residues determined to interact directly with human FcRn includes Ile253, Ser254, Lys288, Thr307, Gln311, Asn434 and His435. Switches at any of these positions described in this section may enable increased serum half-life and/or altered effector properties of antigen binding proteins of the invention.

Antigen binding proteins of the present invention may have amino acid modifications that increase the affinity of the constant domain or fragment thereof for FcRn. Increasing the half-life of therapeutic and diagnostic IgG's and other bioactive molecules has many benefits including reducing the amount and/or frequency of dosing of these molecules. In one embodiment there is therefore provided an antigen binding according to the invention provided herein or a fusion protein comprising all or a portion (an FcRn binding portion) of an IgG constant domain having one or more of these amino acid modifications and a non-IgG

protein or non-protein molecule conjugated to such a modified IgG constant domain, wherein the presence of the modified IgG constant domain increases the in vivo half life of the antigen binding protein.

PCT Publication No. WO 00/42072 discloses a polypeptide comprising a variant Fc region with altered FcRn binding affinity, which polypeptide comprises an amino acid modification at any one or more of amino acid positions 238, 252, 253, 254, 255, 256, 265, 272, 286, 288, 303, 305, 307, 309, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 386,388, 400, 413, 415, 424,433, 434,435, 436, 439, and 447 of the Fc region, wherein the numbering of the residues in the Fc region is that of the EU index (Kabat et al).

PCT Publication No. WO 02/060919 A2 discloses a modified IgG comprising an IgG constant domain comprising one or more amino acid modifications relative to a wild-type IgG constant domain, wherein the modified IgG has an increased half-life compared to the half-life of an IgG having the wild-type IgG constant domain, and wherein the one or more amino acid modifications are at one or more of positions 251, 253, 255, 285-290, 308-314, 385-389, and 428-435. Shields et al. (2001, J Biol Chem ; 276:6591-604) used alanine scanning mutagenesis to alter residues in the Fc region of a human IgG1 antibody and then assessed the binding to human FcRn. Positions that effectively abrogated binding to FcRn when changed to alanine include I253, S254, H435, and Y436. Other positions showed a less pronounced reduction in binding as follows: E233-G236, R255, K288, L309, S415, and H433. Several amino acid positions exhibited an improvement in FcRn binding when changed to alanine; notable among these are P238, T256, E272, V305, T307, Q311, D312, K317, D376, E380, E382, S424, and N434. Many other amino acid positions exhibited a slight improvement (D265, N286, V303, K360, Q362, and A378) or no change (S239, K246, K248, D249, M252, E258, T260, S267, H268, S269, D270, K274, N276, Y278, D280, V282, E283, H285, T289, K290, R292, E293, E294, Q295, Y296, N297, S298, R301, N315, E318, K320, K322, S324, K326, A327, P329, P331, E333, K334, T335, S337, K338, K340, Q342, R344, E345, Q345, Q347, R356, M358, T359, K360, N361, Y373, S375, S383, N384, Q386, E388, N389, N390, K392, L398, S400, D401, K414, R416, Q418, Q419, N421, V422, E430, T437, K439, S440, S442, S444, and K447) in FcRn binding.

The most pronounced effect was found for combination variants with improved binding to FcRn. At pH 6.0, the E380A/N434A variant showed over 8-fold better binding to FcRn, relative to native IgG1, compared with 2-fold for E380A and 3.5-fold for N434A. Adding T307A to this effected a 12-fold improvement in binding relative to native IgG1. In one embodiment the antigen binding protein of the invention comprises the E380A/N434A mutations and has increased binding to FcRn.

Dall'Acqua et al. (2002, J Immunol.169: 5171-80) described random mutagenesis and screening of human IgG1 hinge-Fc fragment phage display libraries against mouse FcRn. They disclosed random mutagenesis of positions 251, 252, 254-256, 308, 309, 311, 312, 314, 385-387, 389, 428, 433, 434, and 436. The major improvements in IgG1-human FcRn complex stability occur in substituting residues located in a band across

the Fc-FcRn interface (M252, S254, T256, H433, N434, and Y436) and to lesser extend substitutions of residues at the periphery like V308, L309, Q311, G385, Q386, P387, and N389. The variant with the highest affinity to human FcRn was obtained by combining the M252Y/S254T/T256E and H433K/N434F/Y436H mutations and exhibited a 57-fold increase in affinity relative to the wild-type IgG1. The in vivo behaviour of such a mutated human IgG1 exhibited a nearly 4-fold increase in serum half-life in cynomolgus monkey as compared to wild-type IgG1.

The present invention therefore provides a variant of an antigen binding protein with optimized binding to FcRn. In a preferred embodiment, the said variant of an antigen binding protein comprises at least one amino acid modification in the Fc region of said antigen binding protein, wherein said modification is selected from the group consisting of 226, 227, 228, 230, 231, 233, 234, 239, 241, 243, 246, 250, 252, 256, 259, 264, 265, 267, 269, 270, 276, 284, 285, 288, 289, 290, 291, 292, 294, 297, 298, 299, 301, 302, 303, 305, 307, 308, 309, 311, 315, 317, 320, 322, 325, 327, 330, 332, 334, 335, 338, 340, 342, 343, 345, 347, 350, 352, 354, 355, 356, 359, 360, 361, 362, 369, 370, 371, 375, 378, 380, 382, 384, 385, 386, 387, 389, 390, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401 403, 404, 408, 411, 412, 414, 415, 416, 418, 419, 420, 421, 422, 424, 426, 428, 433, 434, 438, 439, 440, 443, 444, 445, 446 and 447 of the Fc region as compared to said parent polypeptide, wherein the numbering of the amino acids in the Fc region is that of the EU index in Kabat.

In a further aspect of the invention the modifications are M252Y/S254T/T256E.
Additionally, various publications describe methods for obtaining physiologically active molecules whose half-lives are modified either by introducing an FcRn-binding polypeptide into the molecules (WO 97/43316; U.S. Patent N° 5,869,046; U.S. Patent N° 5,747,035; WO 96/32478; WO 91/14438) or by fusing the molecules with antibodies whose FcRn-binding affinities are preserved but affinities for other Fc receptors have been greatly reduced (WO 99/43713) or fusing with FcRn binding domains of antibodies (WO 00/09560; U.S. Patent N°4,703,039).

Additionally, methods of producing an antigen binding protein with a decreased biological half-life are also provided. A variant IgG in which His435 is mutated to alanine results in the selective loss of FcRn binding and a significantly reduced serum half-life (Firan et al. 2001, International immunology 13:993). U.S. Pat. No. 6,165,745 discloses a method of producing an antigen binding protein with a decreased biological half-life by introducing a mutation into the DNA segment encoding the antigen binding protein. The mutation includes an amino acid substitution at position 253, 310, 311, 433, or 434 of the Fc-hinge domain.

The invention also relates to a variant of any specific antigen binding construct sequence disclosed herein, such as epitope binding domain sequence, e.g. a dAb sequence, or the sequence of the whole antigen binding construct. Suitably the variant comprises an amino acid sequence that has at least 70%, or at least 75%, or at least 80% or at least 85% or at least 90% or at least 95%, at least 96%, at least 97%, at least 98% or

at least 99% sequence identity to the specified sequence, suitably whilst substantially retaining the binding properties of the parent sequence, or at least one binding property where the parent is multi-specific, such as some binding affinity for a VEGF, such as VEGF A. Generally any variant will have at least 30% of the binding affinity of the parent, suitably at least 40%, 50%, 60%, 70, 80%, 90% or more, and suitably 100% of the parent sequence (or more).

In one aspect the antigen binding construct may be varied by 1, 2, 3, or 4 amino acids, as long as the antigen binding construct is capable of binding to a VEGF, such as VEGF A.

In one aspect the epitope binding domain sequence comprises a CDR or CDRs from a domain antibody (dAb). CDRs of any sequence identified or referred to herein may be varied by 1, 2, 3, or 4 amino acids, as long as the antigen binding construct is capable of binding to a VEGF such as VEGF-A.

CDRs are defined as the complementarity determining region amino acid sequences of an antigen binding protein. These are the hypervariable regions of immunoglobulin heavy and light chains.

Throughout this specification, amino acid residues in variable domain sequences are numbered according to the Kabat numbering convention, unless otherwise specified. For further information, see Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987).

The table below,(Table 1), represents one definition using each numbering convention for each CDR or binding unit. The Kabat numbering scheme is used in Table 1 to number the variable domain amino acid sequence. It should be noted that some of the CDR definitions may vary depending on the individual publication used.

Table 1: Numbering Convention for CDRs

	Kabat CDR	Chothia CDR	AbM CDR	Contact CDR	Minimum binding unit
H1	31-35/35A/35B	26-32/33/34	26-35/35A/35B	30-35/35A/35B	31-32
H2	50-65	52-56	50-58	47-58	52-56
H3		95-102	95-102	93-101	95-101
L1	24-34	24-34	24-34	30-36	30-34
L2	50-56	50-56	50-56	46-55	50-55

L3	89-97	89-97	89-97	89-96	89-96

In one aspect, the present invention provides antigen binding constructs comprising two dAbs, wherein each dAb is independently selected and comprises:

i) one or more of CDR sequences SEQ ID No. 111, 112 and 113;

ii) one or more of CDR sequences SEQ ID No. 114, 115 and 116;

iii) one or more of CDR sequences SEQ ID No. 117, 118 and 119;

iv) one or more of CDR sequences SEQ ID No. 120, 121 and 122;

v) one or more of CDR sequences SEQ ID No. 123, 124 and 125;

vi) one or more of CDR sequences SEQ ID No. 126, 127 and 128; or

vii) one or more of CDR sequences SEQ ID No. 129, 130 and 131.

In a further aspect, the present invention provides antigen binding constructs comprising two dAbs, wherein the dAb attached to the N-terminus of the Fc region of an antibody comprises one or more CDR sequences SEQ ID No. 114, 115 and 116, and the dAb attached to the C-terminus of the Fc region of an antibody comprises one or more CDR sequences SEQ ID No. 117, 118 and 119.

In a yet further aspect, the present invention provides antigen bindin constructs comprising one dAb, which comprises:

i) one or more of CDR sequences SEQ ID No. 117, 118 and 119;

ii) one or more of CDR sequences SEQ ID No. 120, 121 and 122;

iii) one or more of CDR sequences SEQ ID No. 123, 124 and 125;

iv) one or more of CDR sequences SEQ ID No. 126, 127 and 128; or

v) one or more of CDR sequences SEQ ID No. 129, 130 and 131.

In one aspect there is provided an antigen binding construct or dimer which competes with an antigen binding construct or dimer herein described, for example, for binding to a VEGF, such as VEGF A.

In one aspect an antigen binding construct and dimers as described herein are able to compete with Avastin for binding to VEGF-A.

It will be understood that any of the antigen-binding constructs and dimers described herein will be capable of neutralising one or more antigens.

The term “neutralises” and grammatical variations thereof as used throughout the present specification in relation to antigen binding constructs of the invention means that a biological activity of the target is reduced, either totally or partially, in the presence of the antigen binding constructs of the present invention

in comparison to the activity of the target in the absence of such antigen binding constructs. Neutralisation may be due to but not limited to one or more of blocking ligand binding, preventing the ligand activating the receptor, down regulating the receptor or affecting effector functionality.

Methods of assessing neutralisation, for example, by assessing the decreased binding between the ligand and its receptor in the presence of neutralising antigen binding construct are known in the art, and include receptor binding assays (see Examples 7, 17, 26, 41 and 50 herein) and rabbit retinal leakage model (see Examples 9 and 42 herein).

The invention also relates to a polynucleotide sequence encoding an antigen binding construct of the invention, or encoding a part of such a construct such as an epitope binding domain sequence. Suitably the polynucleotide encodes a polypeptide able to bind to a VEGF, such as VEGF-A. The invention also relates to a polynucleotide sequence encoding an antigen having at least 70% sequence identity to that antigen binding construct or portion thereof, such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%. In one aspect the antigen binding construct or portion thereof is able to bind to a VEGF, such as VEGF-A and prevent or treat in whole or in part disease associated with a VEGF, such as VEGF-A signalling.

The invention also relates to a polynucleotide sequences having at least 70% sequence identity to specific polynucleotide sequences disclosed herein, such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 971%, at least 98%, at least 99% identity.

The antigen binding constructs of the present invention may be produced by transfection of a host cell with an expression vector comprising the coding sequence for the antigen binding construct of the invention. An expression vector or recombinant plasmid is produced by placing these coding sequences for the antigen binding construct in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antigen binding construct. In certain embodiments this second expression vector is identical to the first except insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the coding sequences for the two antigen binding constructs that form a homodimer or heterodimer may reside on a single vector, for example in two expression cassettes in the same vector.

In one aspect, the present invention relates to a recombinant transformed or transfected host cell comprising one or more polynucleotide sequences encoding an antigen binding construct, homodimer or heterodimer as herein described.

A selected host cell can be co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention.. The transfected cell is then cultured by conventional techniques to produce the engineered antigen binding construct of the invention, homodimer or heterodimer. The antigen binding construct, homodimer or heterodimer is screened from culture by appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other antigen binding construct, homodimers or heterodimers as disclosed herein.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors may be used, such as pUC19. Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the antigen binding constructs, homodimers or heterodimers of the present invention. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, cells from various strains of E. coli may be used for replication of the cloning vectors and other steps in the construction of antigen binding constructs, homodimers or heterodimers of this invention.

Suitable host cells or cell lines for the expression of the antigen binding constructs, heterodimers, or homodimers of the invention include mammalian cells such as NS0, Sp2/0, CHO (e.g. DG44), COS, HEK, a fibroblast cell (e.g., 3T3), and myeloma cells, for example it may be expressed in a CHO or a myeloma cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook, J., Fritsch, E., Maniatis, T. 1989: Cold Spring harbour Press, Molecular Cloning: Laboratory Manual.

Bacterial cells and where desired strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. Drosophila and Lepidoptera and viral expression systems. See, e.g. Miller et al., Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

In another aspect, the invention relates to a method for the production of an antigen binding construct, homodimer or heterodimer as herein described, which method comprises the step of culturing a host cell and isolating the antigen binding construct, homodimer or heterodimer.

The present invention also provides a method for the production of an antigen binding construct, homodimer or heterodimer as described herein comprising the steps of:

a) culturing a recombinant host cell comprising an expression vector comprising the isolated nucleic acid as described herein, wherein the FUT8 gene encoding alpha-1,6-fucosyltransferase has been inactivated in the recombinant host cell; and

b) recovering the antigen binding protein.

Such methods for the production of antigen binding constructs, heterodimers and homodimers can be performed, for example, using the POTELLIGENT™ technology system available from BioWa, Inc. (Princeton, NJ) which may produce antigen binding constructs, homodimers and heterodimers having enhanced antibody dependent cell mediated cytotoxicity (ADCC) activity that is increased relative to an identical protein produced in a cell with a functional FUT8 gene. Aspects of the POTELLIGENT™ technology system are described in US7214775, US6946292, WO0061739 and WO0231240 all of which are incorporated herein by reference. Those of ordinary skill in the art will also recognize other appropriate systems.

The present invention also provides a method of producing an antigen binding construct, homodimer or heterodimer as described herein comprising the steps of:

a) culturing a recombinant host cell comprising an expression vector comprising an isolated nucleic acid as described herein wherein the expression vector comprises a nucleic acid sequence encoding an Fc domain having both IgG1 and IgG3 Fc domain amino acid residues; and
b) recovering the antigen binding protein.

Such methods for the production of antigen binding constructs, heterodimers and homodimers can be performed, for example, using the COMPLEGENT™ technology system available from BioWa, Inc. (Princeton, NJ) and Kyowa Hakko Kogyo (now, Kyowa Hakko Kirin Co., Ltd.) Co., Ltd. in which a recombinant host cell comprising an expression vector in which a nucleic acid sequence encoding a chimeric Fc domain having both IgG1 and IgG3 Fc domain amino acid residues is expressed to produce an antigen binding construct, heterodimer or homodimer having enhanced complement dependent cytotoxicity (CDC) activity that is increased relative to an otherwise identical protein lacking such a chimeric Fc domain. Aspects of the COMPLEGENT™ technology system are described in WO2007011041 and US20070148165 each of which are incorporated herein by reference. In an alternative embodiment CDC activity may be increased by introducing sequence specific mutations into the Fc region of an IgG chain. Those of ordinary skill in the art will also recognize other appropriate systems.

It will be apparent to those skilled in the art that such modifications may not only be used alone but may be used in combination with each other in order to further enhance effector function.

In one aspect of the present invention there is provided an antigen binding construct, heterodimer or homodimer comprising a heavy chain constant region which comprises a mutated and chimaeric heavy chain constant region, comprising at least one CH2 domain from IgG3 and one CH2 domain from IgG1, wherein the IgG1 CH2 domain has one or more mutations at positions selected from 239 and 332 and 330 (for example the mutations may be selected from S239D and I332E and A330L) such that the antigen binding protein has enhanced effector function, for example wherein it has one or more of the following functions, enhanced ADCC or enhanced CDC, for example wherein it has enhanced ADCC and enhanced CDC. In one aspect the IgG1 CH2 domain has the mutations S239D and I332E.

In an alternative aspect of the present invention there is provided an antigen binding construct, heterodimer or homdimer, comprising a chimaeric heavy chain constant region and which has an altered glycosylation profile. In one such aspect, the heavy chain constant region comprises at least one CH2 domain from IgG3 and one CH2 domain from IgG1 and has an altered glycosylation profile such that the ratio of fucose to mannose is 0.8:3 or less, for example wherein the antigen binding protein is defucosylated so that said antigen binding protein has an enhanced effector function in comparison with an equivalent antigen binding protein with an immunoglobulin heavy chain constant region lacking said mutations and altered glycosylation

profile, for example wherein it has one or more of the following functions, enhanced ADCC or enhanced CDC, for example wherein it has enhanced ADCC and enhanced CDC

In one aspect of the invention, there is provided a method of producing an antigen binding construct, heterodimer or homdimer as described herein which uses the ACCRETAMAB™ technology system available from BioWa, Inc. (Princeton, NJ) which combines the POTELLIGENT™ and COMPLEGENT™ technology systems to produce an antigen binding protein having both ADCC and CDC enhanced activity that is increased relative to an otherwise identical monoclonal antibody lacking a chimeric Fc domain and which has fucose on the oligosaccharide.

Another method of expression of the antigen binding constructs may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

The invention also relates to a method for producing an antigen binding construct as disclosed herein wherein the amino acid sequence of an antigen binding construct or a nucleic acid encoding it, or a part thereof, is designed using a computer and wherein the construct exists in silico on the computer.

The invention also provides antigen-binding constructs disclosed herein for use in medicine, for example for use in the manufacture of a medicament for treating diseases associated with a VEGF signalling, such as VEGF-A signalling, such as cancer and ocular diseases such as Diabetic Macular Edema (DME), Wet AMD (Age-related macular degeneration), Diabetic retinopathy, RVO, (retinal vein occlusion), and corneal neovascularisation.

The invention also relates to a method of treating a patient suffering from ocular vascular diseases caused by a VEGF signalling, such as VEGF-A, such as cancer and ocular diseases such as Diabetic Macular Edema (DME), Wet AMD, Diabetic retinopathy, RVO, and corneal neovascularisation, comprising administering a therapeutic amount of an antigen-binding construct of the invention.

The invention also relates to an antigen-binding construct of the invention for the treatment of cancer and ocular diseases such as Diabetic Macular Edema (DME), Wet AMD, Diabetic retinopathy, RVO, and corneal neovascularisation.

The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient. It is envisaged that repeated dosing (e.g. once a week or once

every two weeks) over an extended time period (e.g. four to six months) maybe required to achieve maximal therapeutic efficacy.

The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The antigen binding constructs, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously (s.c.), intrathecally, intraperitoneally, intramuscularly (i.m.), intravenously (i.v.), or intranasally.

In one aspect the administration is for an ocular indication and the administration is by local ocular delivery such as intravitreal, (direct injection into the vitreous of the eye) or is periocular, such as for example is trans-scleral, subconjunctival, sub-tenon, peribulbar, topical, retrobulbar or is delivered to the inferior, superior or lateral rectus muscle. In one aspect the administration is by trans-scleral or topical ocular delivery.

For local administration to the eye, for example by intravitreal injection, the pharmaceutical preparation could be administered in a total volume of up to 100µL, for example, 50-100µL, administered by a single injection by a standard syringe with a needle gauge of 25-30g, such as a 50µl volume administered with a 30g needle. Such formulations if prepared for infrequent administration may contain up to 15% of solid suspension within the liquid volume. The dose of active pharmaceutical in such a formulation would vary but in a preferred aspect could include up to 25-30% of the solid suspension. In a preferred aspect 0.5 -2.5mg of active pharmaceutical is administered perocular administration.

In one aspect the administration uses a sustained or slow-release delivery system such as microparticles or a gel-based system, or a liposome based system or any other system known to those skilled in the art that would allow administration locally to the eye, suitably at frequency reduced when compared to a monthly injection regime. Such regimes may allow delivery less frequently than once a week or once every two weeks, and for example could be once every 4 weeks, once every 2 months or 8 weeks, every 3 months or 12 weeks, every 4 months or 16 weeks, every 5 months or 20 weeks, every 6 months or 24 weeks, every 9 months or 36 weeks, or every 12 months or 52 weeks.. A preferred delivery frequency for the antigen binding constructs, heterodimers and homodimers of the present invention is once every 6 months or 24 weeks.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of an antigen binding construct, homodimer or heterodimer of the invention as an active ingredient in a pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the antigen binding construct, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the antigen binding construct of the invention or a cocktail thereof dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.9%

saline, 0.3% glycine, and the like. These solutions may be made sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antigen binding construct of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an antigen binding construct of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 and preferably 5 mg to about 25 mg of an antigen binding construct of the invention per ml of Ringer's solution. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania. For the preparation of intravenously administrable antigen binding construct formulations of the invention see Lasmar U and Parkins D "The formulation of Biopharmaceutical products", Pharma. Sci.Tech.today, page 129-137, Vol.3 (3rd April 2000), Wang, W "Instability, stabilisation and formulation of liquid protein pharmaceuticals", Int. J. Pharm 185 (1999) 129-188, Stability of Protein Pharmaceuticals Part A and B ed Ahern T.J., Manning M.C., New York, NY: Plenum Press (1992), Akers,M.J. "Excipient-Drug interactions in Parenteral Formulations", J.Pharm Sci 91 (2002) 2283-2300, Imamura, K et al "Effects of types of sugar on stabilization of Protein in the dried state", J Pharm Sci 92 (2003) 266-274,Izutsu, Kkojima, S. "Excipient crystalinity and its protein-structure-stabilizing effect during freeze-drying", J Pharm. Pharmacol, 54 (2002) 1033-1039, Johnson, R, "Mannitol-sucrose mixtures-versatile formulations for protein lyophilization", J. Pharm. Sci, 91 (2002) 914-922.
Ha,E Wang W, Wang Y.j. "Peroxide formation in polysorbate 80 and protein stability", J. Pharm Sci, 91, 2252-2264,(2002) the entire contents of which are incorporated herein by reference and to which the reader is specifically referred.

It is preferred that the therapeutic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose will be determined readily by those of skill in the art. Suitable doses may be calculated for patients according to their weight, for example suitable doses may be in the range of 0.01 to 20mg/kg, for example 0.1 to 20mg/kg, for example 1 to 20mg/kg, for example 10 to 20mg/kg or for example 1 to 15mg/kg, for example 10 to 15mg/kg. To effectively treat conditions of use in the present invention in a human, suitable doses may be within the range of 0.01 to 1000

mg, for example 0.1 to 1000mg, for example 0.1 to 500mg, for example 500mg, for example 0.1 to 100mg, or 0.1 to 80mg, or 0.1 to 60mg, or 0.1 to 40mg, or for example 1 to 100mg, or 1 to 50mg, of an antigen binding construct of this invention, which may be administered parenterally, for example subcutaneously, intravenously or intramuscularly. Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician.

The antigen binding constructs described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

There are several methods known in the art which can be used to generate epitope-binding domains of use in the present invention.

In one example, the methods employ a display system that links the coding function of a nucleic acid and physical, chemical and/or functional characteristics of the polypeptide encoded by the nucleic acid. Such a display system can comprise a plurality of replicable genetic packages, such as bacteriophage or cells (bacteria). The display system may comprise a library, such as a bacteriophage display library. Bacteriophage display is an example of a display system.

The term "library" refers to a mixture of heterogeneous polypeptides or nucleic acids. The library is composed of members, each of which has a single polypeptide or nucleic acid sequence. To this extent, "library" is synonymous with "repertoire." Sequence differences between library members are responsible for the diversity present in the library. The library may take the form of a simple mixture of polypeptides or nucleic acids, or may be in the form of organisms or cells, for example bacteria, viruses, animal or plant cells and the like, transformed with a library of nucleic acids. In one example, each individual organism or cell contains only one or a limited number of library members. Advantageously, the nucleic acids are incorporated into expression vectors, in order to allow expression of the polypeptides encoded by the nucleic acids. In a one aspect, therefore, a library may take the form of a population of host organisms, each organism containing one or more copies of an expression vector containing a single member of the library in nucleic acid form which can be expressed to produce its corresponding polypeptide member. Thus, the population of host organisms has the potential to encode a large repertoire of diverse polypeptides.

A number of suitable bacteriophage display systems (*e.g.*, monovalent display and multivalent display systems) have been described. (See, *e.g.*, Griffiths *et al.*, U.S. Patent No. 6,555,313 B1 (incorporated herein by reference); Johnson *et al.*, U.S. Patent No. 5,733,743 (incorporated herein by reference); McCafferty *et al.*, U.S. Patent No. 5,969,108 (incorporated herein by reference); Mulligan-Kehoe, U.S. Patent No. 5,702,892 (Incorporated herein by reference); Winter, G. *et al.*, *Annu. Rev. Immunol. 12*:433-455 (1994); Soumillion, P. *et al., Appl. Biochem. Biotechnol.* 47(2-3):175-189 (1994); Castagnoli, L. *et al., Comb. Chem. High Throughput*

Screen, 4(2):121-133 (2001).) The peptides or polypeptides displayed in a bacteriophage display system can be displayed on any suitable bacteriophage, such as a filamentous phage (*e.g.*, fd, M13, F1), a lytic phage (*e.g.*, T4, T7, lambda), or an RNA phage (*e.g.*, MS2), for example.

When a display system (*e.g.*, a system that links coding function of a nucleic acid and functional characteristics of the peptide or polypeptide encoded by the nucleic acid), such as phage display, is used in the methods described herein, eg in the selection of a dAb or other epitope binding domain, it is frequently advantageous to amplify or increase the copy number of the nucleic acids that encode the selected peptides or polypeptides. This provides an efficient way of obtaining sufficient quantities of nucleic acids and/or peptides or polypeptides for additional rounds of selection, using the methods described herein or other suitable methods, or for preparing additional repertoires (*e.g.*, affinity maturation repertoires). Nucleic acids can be amplified using any suitable methods, such as by phage amplification, cell growth or polymerase chain reaction.

Generally, a library of phage that displays a repertoire of peptides or phage polypeptides, as fusion proteins with a suitable phage coat protein (*e.g.*, fd pIII protein), is produced or provided. The fusion protein can display the peptides or polypeptides at the tip of the phage coat protein, or if desired at an internal position. For example, the displayed peptide or polypeptide can be present at a position that is amino-terminal to domain 1 of pIII. (Domain 1 of pIII is also referred to as N1.) The displayed polypeptide can be directly fused to pIII (*e.g.*, the N-terminus of domain 1 of pIII) or fused to pIII using a linker. If desired, the fusion can further comprise a tag (*e.g.*, myc epitope, His tag). Libraries that comprise a repertoire of peptides or polypeptides that are displayed as fusion proteins with a phage coat protein,can be produced using any suitable methods, such as by introducing a library of phage vectors or phagemid vectors encoding the displayed peptides or polypeptides into suitable host bacteria, and culturing the resulting bacteria to produce phage (*e.g.*, using a suitable helper phage or complementing plasmid if desired). The library of phage can be recovered from the culture using any suitable method, such as precipitation and centrifugation.

The display system can comprise a repertoire of peptides or polypeptides that contains any desired amount of diversity. For example, the repertoire can contain peptides or polypeptides that have amino acid sequences that correspond to naturally occurring polypeptides expressed by an organism, group of organisms, desired tissue or desired cell type, or can contain peptides or polypeptides that have random or randomized amino acid sequences. If desired, the polypeptides can share a common core or scaffold. For example, all polypeptides in the repertoire or library can be based on a scaffold selected from protein A, protein L, protein G, a fibronectin domain, an anticalin, CTLA4, a desired enzyme (*e.g.*, a polymerase, a cellulase), or a polypeptide from the immunoglobulin superfamily, such as an antibody or antibody fragment (*e.g.*, an antibody variable domain). The polypeptides in such a repertoire or library can comprise defined regions of random or randomized amino acid sequence and regions of common amino acid sequence. In

certain embodiments, all or substantially all polypeptides in a repertoire are of a desired type, such as a desired enzyme (*e.g.*, a polymerase) or a desired antigen-binding fragment of an antibody (*e.g.*, human V_H or human V_L). In some embodiments, the polypeptide display system comprises a repertoire of polypeptides wherein each polypeptide comprises an antibody variable domain. For example, each polypeptide in the repertoire can contain a V_H, a V_L or an Fv (*e.g.*, a single chain Fv).

Amino acid sequence diversity can be introduced into any desired region of a peptide or polypeptide or scaffold using any suitable method. For example, amino acid sequence diversity can be introduced into a target region, such as a complementarity determining region of an antibody variable domain or a hydrophobic domain, by preparing a library of nucleic acids that encode the diversified polypeptides using any suitable mutagenesis methods (*e.g.*, low fidelity PCR, oligonucleotide-mediated or site directed mutagenesis, diversification using NNK codons) or any other suitable method. If desired, a region of a polypeptide to be diversified can be randomized.

The size of the polypeptides that make up the repertoire is largely a matter of choice and uniform polypeptide size is not required. The polypeptides in the repertoire may have at least tertiary structure (form at least one domain).

An epitope binding domain or population of domains can be selected, isolated and/or recovered from a repertoire or library (*e.g.*, in a display system) using any suitable method. For example, a domain is selected or isolated based on a selectable characteristic (*e.g.*, physical characteristic, chemical characteristic, functional characteristic). Suitable selectable functional characteristics include biological activities of the peptides or polypeptides in the repertoire, for example, binding to a generic ligand (*e.g.*, a superantigen), binding to a target ligand (*e.g.*, an antigen, an epitope, a substrate), binding to an antibody (*e.g.*, through an epitope expressed on a peptide or polypeptide), and catalytic activity. (See, *e.g.*, Tomlinson *et al.*, WO 99/20749; WO 01/57065; WO 99/58655.)

The members of the immunoglobulin superfamily all share a similar fold for their polypeptide chain. For example, although antibodies are highly diverse in terms of their primary sequence, comparison of sequences and crystallographic structures has revealed that, contrary to expectation, five of the six antigen binding loops of antibodies (H1, H2, L1, L2, L3) adopt a limited number of main-chain conformations, or canonical structures (Chothia and Lesk (1987) *J. Mol. Biol.*, **196**: 901; Chothia *et al.* (1989) *Nature*, **342**: 877). Analysis of loop lengths and key residues has therefore enabled prediction of the main-chain conformations of H1, H2, L1, L2 and L3 found in the majority of human antibodies (Chothia *et al.* (1992) *J. Mol. Biol.*, **227**: 799; Tomlinson *et al.* (1995) *EMBO J.*, **14**: 4628; Williams *et al.* (1996) *J. Mol. Biol.*, **264**: 220). Although the H3 region is much more diverse in terms of sequence, length and structure (due to the use of D segments), it also forms a limited number of main-chain conformations for short loop lengths which depend on the length

and the presence of particular residues, or types of residue, at key positions in the loop and the antibody framework (Martin *et al.* (1996) *J. Mol. Biol.*, **263**: 800; Shirai *et al.* (1996) *FEBS Letters*, **399**: 1).

The dAbs are advantageously assembled from libraries of domains, such as libraries of V_H domains and/or libraries of V_L domains. In one aspect, libraries of domains are designed in which certain loop lengths and key residues have been chosen to ensure that the main-chain conformation of the members is known. Advantageously, these are real conformations of immunoglobulin superfamily molecules found in nature, to minimise the chances that they are non-functional, as discussed above. Variations may occur at a low frequency, such that a small number of functional members may possess an altered main-chain conformation, which does not affect its function.

Where several known main-chain conformations or a single known main-chain conformation has been selected, dAbs may be constructed by varying the binding site of the molecule in order to generate a repertoire with structural and/or functional diversity. This means that variants are generated such that they possess sufficient diversity in their structure and/or in their function so that they are capable of providing a range of activities.

In one aspect, the present invention include sequences which are substantially identical, for example sequences which are at least 90% identical, for example which are at least 91%, or at least 92%, or at least 93%, or at least 94% or at least 95%, or at least 96%, or at least 97% or at least 98%, or at least 99% identical to the sequences described herein.

For nucleic acids, the term “substantial identity” indicates that two nucleic acids, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate nucleotide insertions or deletions, in at least about 80% of the nucleotides, usually at least about 90% to 95%, and more preferably at least about 98% to 99.5% of the nucleotides. Alternatively, substantial identity exists when the segments will hybridize under selective hybridization conditions, to the complement of the strand.

For nucleotide and amino acid sequences, the term “identical" indicates the degree of identity between two nucleic acid or amino acid sequences when optimally aligned and compared with appropriate insertions or deletions. Alternatively, substantial identity exists when the DNA segments will hybridize under selective hybridization conditions, to the complement of the strand.

The percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = no. of identical positions/total no. of positions times 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences

can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

By way of example, a polynucleotide sequence of the present invention may be identical to a reference sequence, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in the reference sequence by the numerical percent of the respective percent identity (divided by 100) and subtracting that product from said total number of nucleotides in the reference sequence, or:
$nn \leq xn - (xn \bullet y)$, wherein nn is the number of nucleotide alterations, xn is the total number of nucleotides in the reference sequence, and y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100% identity, and wherein any non-integer product of xn and y is rounded down to the nearest integer prior to subtracting it from xn. Alterations of the polynucleotide sequence of the reference sequence may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Similarly, in another example, a polypeptide sequence of the present invention may be identical to a reference sequence, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a

given % identity is determined by multiplying the total number of amino acids in the polypeptide sequence encoded by the reference sequence by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in the polypeptide reference sequence or:

$$na \leq xa - (xa \bullet y),$$

wherein na is the number of amino acid alterations, xa is the total number of amino acids in the polypeptide sequence and y is, for instance, 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of xa and y is rounded down to the nearest integer prior to subtracting it from xa.

The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine study, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims. All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

The term "or combinations thereof" as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations thereof is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB.

Examples

It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention.

Throughout the examples references are made to dAb-Fc, Fc-dAb and dAb-Fc-dAb molecules. The terms “dAb-Fc” and “Fc-dAb” are simple references to dimers comprising two antigen binding constructs wherein

each antigen binding construct has a dAb attached to either the N-terminus (dAb-Fc) or C-terminus (Fc-dAb), directly or indirectly through a linker.

Similarly, the term "dAb-Fc-dAb" refers to dimers comprising two antigen binding constructs wherein each antigen binding construct has a dAb attached to the N-terminus and a dAb attached to the C-terminus; directly or indirectly through a linker.

Example 1 – Expression of Vh dAb-Fc molecules

DMS1529, (SEQ ID NO:1 & 29), has been described in W02008/149150/A20. DMS1576, (SEQ ID NO:2 & 30), was generated by site directed mutagenesis from DMS1529 converting amino acid 56 from Y to N. DMS1529 and DMS1576 were manufactured from either CHROMOS pooled (or bulk) transfections or stable polyclonal or monoclonal CHO cell lines using GlaxoSmithKline's generic monoclonal antibody production process platform using a combination of shake flask and stirred tank suspension culture. Bioreactors are monitored and maintained at controlled conditions for agitation speed, dissolved oxygen concentration, pH and temperature. Dissolved oxygen is maintained through the addition of 40% O_2 in N_2 while pH is controlled via automated addition of sodium carbonate and CO_2. The production culture duration is determined from a combination of cell viability and minimum antibody titre. At the end of the production period, the culture in the bioreactor is clarified by depth filtration and sterile filtration to generate a batch of clarified unprocessed bulk (CUB).

Example 2 – Purification of Vh dAb-Fc molecules

DMS1529 and DMS1576 were captured from clarified cell culture supernatant (CUB, Example 1) using affinity chromatography and an automated FPLC purification system. Once loaded, the bound product was washed using a combination of pH neutral aqueous buffers to remove non-specifically bound impurities followed by a low pH elution. Over 90% of bound product was recovered and the pH of the elution pool then adjusted to pH 3.5 for 30 minutes to achieve virus neutralisation after which time the pH was adjusted to pH 4.5. If further purification was required, a further pH adjustment was performed in order to achieve binding on the second column. Following binding, the product was washed in a low conductivity buffer at equivalent pH to further remove non-specifically bound impurities. The purified dAb-Fc was then eluted using a pH and salt shift and collected as a pool before 0.2um filtration and storage. Unless otherwise stated, protein prepared using only the first, affinity column was analysed for VEGF binding in the in vitro assays described in the following examples, however reference will be made when the test material was further purified, i.e. using the 2nd column.

Example 3 – Molecular Analysis by SDS-PAGE and Size-Exclusion Chromatography (SEC)

The molecular integrity, homogeneity and percentage (%) purity of DMS1529 and DMS1576 were analysed by SDS-PAGE, under both reducing and non-reducing conditions, and analytical size-exclusion chromatography (SEC). SDS-PAGE analysis was carried out according to the manufacturer's instructions using the Novex "NuPAGE" system and gels were stained with Instant Blue Protein Stain Solution (Triple Red Ltd). The gels showed band sizes consistent with the predicted molecular mass of the intact mature proteins (~76-79kDa) allowing for the presence of the predicted glycan chains per monomer chain. SEC was carried out using a TSK gel G2000SWXL column (TOSOH, BioScience). A sodium phosphate/sodium chloride based mobile phase at neutral pH was used at a flow rate of 0.5ml/min. The standard sample injection volume of purified protein (at approximately 1mg/ml) was 10ul. The UV absorbance of the column effluent was monitored at 214 & 280nm. The area of all protein related peaks were integrated to determine the purity of the peak relating to the molecules. The proteins were confirmed to be >95% pure target protein by SDS-PAGE and SEC prior to further analysis in biology assays, (data not shown).

Example 4 – VEGF Binding ELISA

The ability of DMS1529 and DMS1576 to bind specifically to $VEGF_{165}$ was determined and compared to that of Bevacizumab (Avastin, clinical drug product sourced from Phillip Chapper & Co. Ltd., UK) by ELISA. An F96 Maxisorp 96 well flat bottom immunoplate (Nunc, Cat No: 439454) was coated with 100ul of 25ug/ml of hVEGF165 (GSK 'in house' source of VEGF made from HEK293 mammalian cells) and incubated at 4°C overnight. The plate was washed six times with PBS containing 0.05% of Tween-20, 200µl of blocking solution (1% BSA in PBS) was added to each well and the plate was incubated for 1h at room temperature. The plate was then washed with 0.05% Tween-20/PBS. 90µl of assay diluents (0.1% BSA, 0.05% Tween-20 in PBS) was added to each well, 10µl of each sample or control (successively diluted, two-fold over a concentration range from 80-0.08ng/ml) were then added across the plate in blocking solution and incubated for 1hr at room temperature. The plate was then washed 0.05% Tween-20/PBS. 100µl of anti-human IgG (Fc specific) HRP (Sigma, Cat No: A0170) diluted at 1:10,000 in 0.1% BSA, 0.05% Tween-20 in PBS was added to appropriate wells. The plate was incubated for 1 hr at room temperature and washed with 0.05% Tween-20/PBS. 100µl of 3,3',5,5'-Tetramethylbenzidine (TMB) liquid substrate system (Sigma T0440) was added to each well. Once sufficient blue colour has developed (expected OD450 of >2.0), the reaction was stopped 15 minutes later by addition of 100µL of 0.25M sulphuric acid (Fisher Scientific J/8410/17). Absorbance was read at 450nm using the SpectraMax Plus384 Microplate Reader (Molecular Devices) using a basic endpoint protocol.

Both DMS1529 and DMS1576 were found to bind specifically in a similar manner to $VEGF_{165}$, and both molecules were shown to be more potent than Bevacizumab (Avastin) as shown by a reduced IC50 value, (**Figure 1**).

Example 5 – VEGF Binding MSD

The ability of DMS1529 and DMS1576 to bind specifically to $VEGF_{165}$ was determined by MSD (Meso Scale Discovery) assay. The MSD data show equivalent binding of DMS1529 and DMS1576 to hVEGF165 after detection with either anti-Vh or anti-Fc reagents.

Example 6 – Binding of DMS1529 and DMS1576 to VEGF using Surface Plasmon Resonance

The binding affinity of the DMS1529 and DMS1576 molecules for $VEGF_{165}$ was determined by surface Plasmon resonance (SPR) using a Biacore T100 (GE Healthcare), **Example 6A**, and ProteOn XPR36 protein interaction array system (BioRad) (data not shown).

Example 6A – Binding of DMS1529 and DMS1576 to VEGF using Biacore

Protein A was immobilised on a C1 chip by primary amine coupling and this surface was then used to capture the anti-VEGF constructs. Human recombinant VEGF (sourced 'in house' from transient transfection of HEK293 cells) was used as the analyte at 256nM, 64nM, 16nM, 4nM, 1nM, 0.25nM and 0.0625nM. All binding curves were double referenced with a buffer injection (i.e. 0nM) and the data was fitted both to the 1:1 model (and to the bivalent model (inherent to the T100. Regeneration was carried out using 100mM H_3PO_4. The run was carried out at room temperature, using HBS-EP as the running buffer. For the DMS1529 and DMS1576 reliable kinetics could not be obtained due to the poor fitting of the analysis model to the experimental data, (but estimates could be made for association and dissociation constants and these are summarized in Table 2A.

Both Biacore and ProteOn data show that DMS1529 and DMS1576 have comparable binding kinetics to $VEGF_{165}$ using Surface Plasmon Resonance.

Table 2A: Biacore analysis of the binding kinetics of DMS1529 and DMS1576 to human $VEGF_{165}$ to determine ka, kd and KD

	1:1 binding model			Bivalent analyte model		
	ka (M-1.s-1)	kd (s-1)	KD (M)	ka (M-1.s-1)	kd (s-1)	KD (M)
DMS1529	5.23E+05	3.13E-04	5.99E-10	4.89E+05	4.94E-04	1.01E-09
DMS1576	4.60E+05	3.82E-04	8.30E-10	7.95E+05	6.50E-04	8.17E-10

Example 7 – VEGF R1 & R2 Receptor Binding Assay

The potencies of DMS1529 and DMS1576 were analysed in the VEGF receptor binding assay in comparison to that of Bevacizumab (Avastin). This assay measures the binding of VEGF165 to either VEGF R1 or VEGF R2 and the ability of the test molecules to block this interaction. A MSD standard bind 96 well plate (L11XA-3) was coated with 0.25µg/ml VEGF R1 (R&D Systems 321-FL) or VEGF R2 (R&D 357-KD) in bicarbonate buffer (50µl/well), covered with a plate sealer and incubated overnight at 4°C. The next day the MSD plate was washed 3x 300µl/well with Tris wash buffer and blotted over a pad of tissue to remove excess wash buffer from the wells. The MSD plate was then blocked with 3% BSA in PBS (250µl/well) and incubated shaking (750 RPM) at room temperature for 1 hour. The MSD plate was washed again before the addition of a 2x concentration of anti-VEGF molecule (25µl/well) and incubated with shaking (750 RPM) at room temperature for 10 minutes before the addition of a 2x concentration of rhVEGF, 25µl/well, R&D Systems (293-VE/CF, made in insect cells using Baculovirus) or a GSK 'in house' source of VEGF (made from HEK293 mammalian cells, latter data not shown except Table 3A). The anti-VEGF molecules and the VEGF were prepared using 0.1% BSA in PBS. The initial assay was performed with a step in which the anti-VEGF molecule was pre-incubated with VEGF. The pre-incubations were prepared by adding an equal volume of a 2x concentration of anti-VEGF molecule to an equal volume of a 2x concentration of VEGF (R&D, 293-VE/CF) for 30 minutes at room temperature. The final VEGF concentration used was 10ng/ml. No VEGF and VEGF alone controls were also included. The MSD plate was incubated with shaking (750 RPM) at room temperature for 2 hours after which it was washed again before the addition of the detection reagent (50µL/well, goat anti-human VEGF biotinylated antibody – R&D Systems BAF293) at 0.25µg/ml in 1% BSA in PBS and incubated with shaking (750 RPM) at room temperature for 1 hour. The MSD plate was washed again before the addition of the streptavidin sulfo-TAG (50µl/well, MSD R32AD-1) at 2µg/ml in 1% BSA in PBS and incubated with shaking (750 RPM) at room temperature for 30 minutes. Prior to measurement of the electrochemiluminescence in a MSD Sector Imager 6000, the MSD plate was washed and 150µl/well of 2x Read Buffer T (MSD R92TC-1) was added. Curve fitting and EC50 calculations were performed using GraphPad Prism. The ability of DMS1529, DMS1576 and Bevacizumab (Avastin) to inhibit VEGF binding to VEGFR1 and VEGFR2 was determined as described. The EC50 values are listed in Table 3A.

A second assay was performed whereby the anti-VEGF agent and the VEGF were not pre-incubated prior to the addition to the VEGF Receptor coated MSD plate. This assay was carried out just comparing DMS1576 and Bevacizumab (Avastin) and only used VEGF sourced from R&D Systems, (293-VE/CF). The ability of DMS1576 and Bevacizumab (Avastin) to inhibit VEGF binding to VEGFR1 and VEGFR2 was determined as described above but without the pre-incubation step. The EC50 values are listed in Table 3B.

From the data in Table 3A, both DMS1529 and DMS1576 appear to have similar EC50 values and these are almost ten-fold lower (i.e. greater potency) than the EC50 values for Bevacizumab (Avastin) against both VEGF binding to VEGFR1 and VEGFR2. All anti-VEGF molecules are more potent at binding R&D Systems VEGF compared to 'in-house' HEK293 produced VEGF for both VEGFR1 and VEGFR2 binding assays and are more potent in the assays where pre-incubation with VEGF occurs. Since DMS1576 maintained the relative EC50 value compared to that for Bevacizumab (Avastin) against both VEGFR1 and VEGFR2 in the absence of pre-incubation with VEGF, this simplified assay type was taken forward for the analysis of new molecules. Note that in all RBA assays although both DMS1529 and DMS1576 appear more potent than Bevacizumab (Avastin), unlike Bevacizumab (Avastin) neither DMS1529 nor DMS1576 seem to quite reach 100% inhibition in these assays, (data not shown).

Table 3 EC50 values from VEGFR RBAs

(A) RBA with pre-incubation of anti-VEGF & VEGF reagent

In-house VEGF and VEGF sourced from R&D Systems (Baculovirus produced)

		EC50s (g/mL)			EC50s (nM)		
Receptor	VEGF Source	Avastin	DMS1529 Lead	DMS1576 NTY	Avastin	DMS1529 Lead	DMS1576 NTY
VEGFR1	R&D	1.12E-07	5.09E-09	7.01E-09	0.747	0.068	0.093
VEGFR1	GSK	3.07E-07	4.97E-08	5.91E-08	2.048	0.663	0.789
VEGFR2	R&D	1.06E-07	4.65E-09	5.70E-09	0.706	0.062	0.076
VEGFR2	GSK	2.35E-07	3.64E-08	4.38E-08	1.569	0.486	0.583

(B) Modified RBA (without pre-incubation with anti-VEGF agent)

VEGF sourced from R&D Systems (Baculovirus produced).

		EC50 (nM)
VEGF R1	Avastin	3.173
	DMS1576	0.389
VEGF R2	Avastin	5.607
	DMS1576	0.263

Example 8 - Human Umbilical Cord Endothelial Cell (HUVEC) Proliferation assay

DMS1529 and DMS1576 were assayed for their ability to suppress proliferation of human umbilical vein endothelial cells compared to that of Bevacizumab (Avastin). The assay measures the extent of endothelial cell proliferation induced by a defined concentration of $VEGF_{165}$ and the ability of VEGF antagonists to block this effect. HUVECs were seeded at 5000 cells per well in 96-well gelatine-coated plates, leaving outer wells free of cells, and incubated for several hours to permit adherence. Test molecules were assayed at equimolar concentrations (max $3.33x10^{-08}$M) with a 2-fold serial dilution, each in triplicate. The $VEGF_{165}$ was prepared in basal medium to achieve 75ng/ml final concentration. Medium was removed manually from the cell monolayers and 50µl basal media was added to prevent the cells from drying out. 25µl $VEGF_{165}$-containing medium and 25µl basal medium or test antibody-containing medium was added as appropriate. Cells were incubated for 72hrs, after which time the total number of cells was determined using Cell Titre Glo. Treatment of HUVECs with $VEGF_{165}$ resulted in the expected increase in the total number of cells after 72hrs, when compared with $VEGF_{165}$-untreated cells (data not shown). This VEGF-mediated increase is interpreted as representing the cumulative effects of VEGF on both HUVEC proliferation and prevention of HUVEC cell death. The test compounds were independently assessed on individual plates against the comparator molecule, Bevacizumab (Avastin).

DMS1529 was evaluated in a two separate assays. The data suggest that DMS1529 is only able to inhibit HUVEC proliferation by ~50%, cf ~100% for Bevacizumab (Avastin) and the best fit curve suggests that DMS1529 has a higher IC50 (i.e. is less potent) than Bevacizumab (Avastin) in this assay. DMS1576 has been evaluated on several occasions in the HUVEC assay. In one sample data set, the data suggest that similar to DMS1529, DMS1576 is only able to inhibit HUVEC proliferation by ~50%, cf ~100% for Bevacizumab (Avastin); and the best fit curve suggests that DMS1576 has a higher IC50 (i.e. is less potent) than Bevacizumab (Avastin) in this assay. However, two other data sets suggest a smaller shortfall in % maximum inhibition and IC50 of DMS1576 cf Bevacizumab (Avastin), (data not shown).

Example 9 – Performance in VEGF induced blood-retinal breakdown: rabbit retinal leakage model

DMS1529 and DMS1576 were tested in a human $VEGF_{165}$ (R&D Systems), induced blood-retinal breakdown, (BRB), model in the rabbit eye and compared against Bevacizumab (Avastin), Ranibizumab (Lucentis), and Kenacort (Tramcinalone). All sources of Bevacizumab, Ranibizumab and Kenacort were obtained from clinical sources as described previously, Phillip Chapper & Co. Ltd, UK. The model has been described in some detail in the literature and is also known as the 'Edelman model' (Edelman JL, Lutz D, Castro MR, Corticosteroids inhibit VEGF-induced vascular leakage in a rabbit model of blood-retinal and blood-aqueous barrier breakdown, Exp Eye Res. 2005 Feb; 80(2):249-58).

The aim of this study was to evaluate the potency of DMS1529 and DMS1576, at two doses: High, (H), and Low, (L), in reducing the retinal vascular leakage in a recombinant humanVEGF165-induced blood retinal barrier breakdown model in rabbits. The high (H) and low (L) doses were based upon a molar equivalent dosing to Ranibizumab (Lucentis),. The low dose, (L), was one third of the high dose. Bevacizumab (Avastin) was also dosed at a scaled down dose from the dose used in the clinic for ocular indications. The dosing and injection schedule is shown in Table 4A.

In brief, all molecules were buffer exchanged using PD-10 Desalting Columns (GE Healthcare) into 10mM Histidine HCl, 10% α-α trehalose dehydrate, 0.01% PolySorbate 80, pH 5.5, and concentrated to the desired concentration using Vivaspin 20, molecular weight cut off 5000Da, spin concentrations (Sartorius Stedim Biotech), both were used according to manufacturer's instructions. Samples were frozen at -80°C, and shipped on dry ice, after testing to confirm stability, functional activity and uniformity after this process as described in the aforementioned examples (data not shown).

Ninety-eight (98) GD79B pigmented rabbits were randomly divided into nine (9) groups of ten (10) animals and one (1) group of eight (8) animals (used for Kenacort control). Each group was subdivided into 2 groups corresponding to 2 experimental sets. Test items, reference or control items were administered by intravitreal injection (IVT, 50 µL) into the right eyes on Day -7. Left eyes remained untreated and served as a negative control. On Day 0, right eyes were induced for blood retinal barrier (BRB) breakdown with a single intravitreal injection of 500ng rhVEGF$_{165}$ (vascular permeability inducer). Sodium fluorescein was intravenously injected to all groups 47h after the VEGF challenge (Day 2). Within 10 min after the injection of the tracer a retinal angiography was performed on right eye and pictures were taken. Ocular fluorescein contents in the vitreoretinal compartment were measured 1h later using non-invasive scanning ocular fluorophotometry. A right eye/left eye fluorescein content (AUC) Rt ratio was determined for retinal permeability evaluation. At the end of the evaluation period (Day 2), animals were euthanized and right eyes of all animals were enucleated. Snap-frozen eyeballs and aqueous humor samples were stored at -80°C until shipment to the sponsor, GSK. Fluorescein angiograms were collected for qualitative assessment. The compounds remained masked upon GSK's request. The administration schedule for this is summarised in Table 4A.

Intravitreally injected VEGF induced a breakdown of the BRB, which was blocked by treatment with compounds masked labelled E: Bevacizumab (Avastin), H: Ranibizumab (Lucentis, high dose) and I: Ranibizumab (Lucentis, low dose), 9 days post injection, with an efficacy similar to that of the marketed reference (Kenacort®). Low values of Rt ratio of vitreoretinal fluorescein contents between right-induced and left eyes were observed (Rt = 1.39 ± 0.77 (n = 9) for compound E, Rt = 1.05 ± 0.52 (n = 10) for compound H and Rt = 1.81 ± 1.76 (n = 10) for compound I. Kenacort-treated group showed a mean Rt ratio close to that of non-induced animals (Rt = 1.15 ± 0.61).

In the masked study, an important retinal vascular leakage was noted in right induced eyes after treatment with compounds A: DMS1576 (high dose), B: DMS1576 (high dose), C: Vehicle, negative control group D: DMS1576 (low dose), F: DMS1529 (low dose) and G: DMS1529 (high dose). Without unmasking of the compounds and comparison to vehicle control compounds A, B, C, D, F and G were clearly less effective than compounds E, H and I.

Unmasked data and statistical analysis demonstrating the inhibition of VEGF induced rabbit retinal leakage is shown in Table 4B. For this analysis the masked groups corresponding to the same molecule and dose were pooled. For the data in Table 4B: P values are shown with and without multiple comparison adjustment, labelled: 'Dunnett' and 'unadjusted' p value respectively. Confidence intervals correspond to unadjusted p value (CI includes ratio of 1 at p>0.05). From the data analysis in Table 4B: DMS1529 (46%) and DMS1576 (41%) at high dose only partially reduced the degree of VEGF induced retinal leakage and at low doses the reduction was even less: DMS1529 (19%) and DMS1576 (25%). Under the same conditions, compounds E: Bevacizumab (Avastin, 75%), H: Ranibizumab (Lucentis, high dose, 82%) and I: Ranibizumab (Lucentis, low dose, 78%), suppressed the VEGF-induced retinal vascular leakage, with an effect similar to the marketed corticoid reference (Kenacort® retard, 85%).

Table 4A: Dosing and injection schedule for inhibition of VEGF induced rabbit retinal leakage by DMS1529, DMS1576 compared to Bevacizumab (Avastin), Ranibizumab (Lucentis) and Kenacort (Triamcinalone).

Group No.	Treatment / compound dosed (right eye)	Dose	Treatment Protocol	Numbers of animals		Induction	Measurements
				Set 1	Set 2		
1	Vehicle	-	50-µL IVT Day -7	5	5	Day0, 500ng/50 µL rhVEGF, (R&D Systems (delivered by intravitreal injection,	*On Day 2*: * Fluorescein leakage quantification in vitreous/retina segment (Fluorotron® Master). * Retinal angiography
2	Lucentis high dose	H		5	5		
3	Lucentis low dose	L		5	5		
4	Avastin	H		5	5		
5	DMS1529 high dose	H		5	5		
6	DMS1529 low dose	L		5	5		
7	DMS1576 high dose	H		5	5		
8	DMS1576 low dose	H		5	5		
9	DMS1576 high dose	H		5	5		

10	Kenacort® Retard	2 000 µg/eye		4	4	IVT)	assessment using Heidelberg's Retinal Angiograph * Eyeballs sampling (except for Kenacort group)

Table 4B: Inhibition of VEGF induced rabbit retinal leakage by DMS1529, DMS1576 compared to Bevacizumab (Avastin), Ranibizumab, (Lucentis) and Kenacort (Triamcinalone)

% reduction vs vehicle	*treatment*	*Ratio vs Vehicle*	*Lower 95% CI*	*Upper 95% CI*	*Unadjusted p value*	*Dunnett p value*
46%	DMS1529H	0.54021	0.29596	0.98606	0.0450	0.2234
19%	DMS1529L	0.80759	0.44463	1.46685	0.4786	0.9788
41%	DMS1576H	0.58638	0.34873	0.98597	0.0442	0.2201
25%	DMS1576L	0.75355	0.41376	1.37237	0.3508	0.9103
75%	Avastin	0.25446	0.13782	0.46984	<.0001	0.0002
85%	Kenacort	0.14853	0.07769	0.28395	<.0001	<.0001
82%	Lucentis H	0.18258	0.10048	0.33175	<.0001	<.0001
78%	Lucentis L	0.21876	0.12005	0.39865	<.0001	<.0001

Example 10 – Cloning of anti-VEGF Vk dAb-Fc and Fc-dAb Molecules

The dAb sequences (SEQ ID NO:97-101, 105-109) were cloned onto the N- or C-terminus of a generic Fc of the human IgG1 isotype in a mammalian expression vector. The dAbs were linked to the Fc using a linker sequence: the N-terminal linker was either AAAS (SEQ ID NO:57 & 76), or TVAAPS (SEQ ID NO:59 & 78) and the C-terminal linker was either ((GS(TVAAPSGS)x3) (SEQ ID NO:66 & 85), or Albumin Domain 3 (SEQ ID NO:71 & 90).

Example 11 – Expression of anti-VEGF Vk dAb-Fc and Fc-dAb Molecules

Expression plasmids encoding the relevant Vk anti-VEGF dAb-Fc and Fc-dAb molecules (listed in SEQ ID NO:3-9, 16-24, 31-37 & 44-52, Table 19) were transiently transfected into HEK293 6E cells and expressed at 500ml scale to produce the antibody fragment molecules. 500ug of plasmid DNA was added to 18ml of OptiMEM (Invitrogen) and separately 666ul of 293fectin was added to 18ml OptiMEM. Both tubes were incubated at room temperature for 5 minutes. The DNA/OptiMEM solution was added slowly to the 293fectin/OptiMEM tube with gentle swirling. The DNA/293fectin transfection complex was then allowed to form for 25 minutes at room temperature. A HEK293E suspension cell culture was diluted to give 0.5x 10^6 cells per ml and the above transfection complex were added slowly to 500ml of the diluted cell culture, with gentle swirling of the culture flask. The flask was then returned to the 37°C, 5% CO_2 incubator, with shaking at 135rpm. 24hrs post-transfection 12.5ml of 20% w/v casein-tryptone was added to the cell culture and incubation was continued as above. 6 days post-transfection, the culture was centrifuged at 5,500xg for 20 minutes to pellet the cells; the supernatant was filtered (0.22um) and analysed for secreted protein expression. Expression levels of 50-100mg/L supernatant were routinely achieved.

Example 12 – Purification of Vk anti-VEGF dAb-Fc and Fc-dAb molecules

The Vk anti-VEGF dAb-Fc and Fc-dAb molecules were affinity purified from the supernatants (see Example 11). 20ml of suspended affinity resin in sodium phosphate buffer (50:50 slurry) was added to 500ml of filtered supernatant; the supernatant/affinity resin mix was rolled gently at +4°c overnight, for ~3h at room temperature, to allow binding to take place. After which time, the resin was allowed to settle and the supernatant carefully poured off. The resin was re-suspended in remaining supernatant and poured into an empty drip column. The supernatant was allowed to pass out of the column, and the resin was then washed with 3 x 10 column volume washes of PBS followed by 4 x column washes of Tris buffer. Elution was carried out using 4 x column volumes of low pH buffer and the eluate collected into a tube containing 1 x column volume of 1M Tris pH 8.0 to neutralize the eluted protein.

Example 13 – Molecular Analysis by SDS-PAGE and Size-Exclusion Chromatography (SEC) of Vk anti-VEGF dAb-Fc and Fc-dAb molecules

The molecular integrity, homogeneity and % purity of the anti-VEGF dAb-Fc and Fc-dAb molecules which had been purified as described in **Example 12** were analysed by SDS-PAGE, following **Example 3**. The gels showed band sizes consistent with the predicted molecular mass of the intact mature protein (from ~76kDa to ~85kDa for dAb-Fc and Fc-dAb molecules respectively), allowing for the presence of the predicted glycan chains per monomer chain. The proteins were confirmed to be >95% pure target protein by SDS-PAGE and SEC prior to further analysis in biology assays. If the dAb-Fc or Fc-dAb molecule was <95% pure a further SEC purification was carried out (see **Example 14**).

Example 14 – Purification by Size-Exclusion Chromatography (SEC) of Vk anti-VEGF dAb-Fc and Fc-dAb molecules

If necessary, preparative size-exclusion chromatography (SEC) was carried out for the Vk dAb-Fc and Fc-dAb molecules using a HiLoad 16/600 Superdex 200 column (GE Healthcare). The mobile phase used was phosphate buffered saline at a flow rate of 0.5ml/min and 0.5-2ml fractions were collected. The UV absorbance of the column effluent was monitored at 214 & 280nm. The fractions collected for the peak corresponding to the elution of the dAb-Fc or Fc-dAb molecule were pooled. The molecular integrity, homogeneity and % purity was again analysed by SDS-PAGE and analytical SEC as described in **Example 13**. The proteins were confirmed to be >95% pure target protein by SDS-PAGE and SEC prior to further analysis in biology assays.

Example 15 – VEGF Binding ELISA of Vk anti-VEGF dAb-Fc and Fc-dAb molecules

The ability of the Vk anti-VEGF dAb-Fc and Fc-dAb molecules to bind specifically to $VEGF_{165}$ was determined by ELISA. This was performed in a similar manner to **Example 4.** All of the anti-VEGF compounds tested were found to bind specifically to $VEGF_{165}$ (data not shown).

Example 16 – Binding of anti-VEGF Vk dAb-Fc and Fc-dAb molecules to VEGF on Biacore

The binding affinity of the anti-VEGF Vk dAb-Fc and Fc-dAb molecules for $VEGF_{165}$ was determined by Surface Plasmon resonance (SPR) using a Biacore T100 in a similar manner to Example 6, but with minor modifications. Protein A was immobilised on a C1 chip by primary amine coupling and this surface was then used to capture the anti-VEGF constructs. Human recombinant $VEGF_{165}$ (sourced 'in house' from transient transfection of HEK293 cells) was used as the analyte at 64nM, 16nM, 4nM, 2nM, 1nM, 0.5nM and 0.25nM. All binding curves were double referenced with a buffer injection (i.e. 0nM) and the data was fitted to 1:1 model inherent to the T100. Regeneration was carried out using 50mM NaOH. The run was carried out at 37°C, using HBS-EP as the running buffer.

The Vk dAb-Fc and Fc-dAb molecules were compared to DMS1576 and Bevacizumab (Avastin). The data shows that the Vk Fc-dAb molecules (DMS30000, DMS30001, DMS30002, DMS30003 and DMS30004) are all improved compared to DMS1576 and the Vk dAb-Fc in terms of binding to VEGF, as determined by Biacore, (see Table 5).

Table 5: Binding of anti-VEGF dAb-Fc and Fc-dAb molecules and Bevacizumab (Avastin) to $VEGF_{165}$

Sample	Temp (°C)	ka	kd	KD (nM)
Bevacizumab	37	Unable to fit data		

(Avastin)		Kinetic constant kd is outside the limits that can be measured by the instrument		
DMS1576	37	Poor fit to 1:1 model		
DMS30005	37	Poor fit to 1:1 model		
DMS30006	37	Poor fit to 1:1 model		
DMS30000	37	1.44E+07	3.34E-05	0.002
DMS30001	37	1.62E+07	3.63E-05	0.002
DMS30002	37	1.25E+07	3.63E-05	0.003
DMS30003	37	1.27E+07	3.84E-05	0.003
DMS30004	37	1.28E+07	4.54E-05	0.004

Example 17 – VEGF R1 & R2 Receptor Binding Assay of anti-VEGF Vk dAb-Fc and Fc-dAb molecules

The potencies of the anti-VEGF Vk dAb-Fc and Fc-dAb molecules were analysed in the VEGF receptor, (R1 and R2), binding assay using the modified method, i.e. with no pre-incubation, described in **Example 7** and were compared to the Vh dAb-Fc, DMS1576 and Bevacizumab (Avastin). The Vk Fc-dAb molecules (DMS30000, DMS30003 and DMS30004) were seen to be more potent (i.e. lower EC50 values, see Tables 6A & 6B), than DMS1576 and Bevacizumab (Avastin) against both VEGFR1 and VEGFR2; whereas the Vk dAb-Fc molecule (DMS30005) was less potent than DMS1576 and similar to Bevacizumab (Avastin) against VEGFR1 and more potent than Bevacizumab (Avastin) against VEGFR2. Against VEGFR1, the data indicate that the inhibition achieved by the Vk Fc-dAb molecules (DMS30000, DMS30003 and DMS30004) are slightly reduced at 87-89% cf the Vh dAb-Fc molecule (DMS1576) and Bevacizumab (Avastin), both ≥90%. The Vk dAb-Fc molecule (DMS30005) is further reduced, 82%. Against VEGFR2, the data indicate that the inhibition achieved by the Vk Fc-dAb molecules (DMS30000, DMS30003 and DMS30004) match that of the Vh dAb-Fc molecule (DMS1576) and Bevacizumab (Avastin), all ≥90%. The Vk dAb-Fc molecule (DMS30005) is slightly reduced, 86%.

Table 6A: EC_{50} values of anti-VEGF compounds compared to Bevacizumab (Avastin) in VEGFR1 Receptor Binding Assay.

Curve fitting and EC_{50} calculations were performed using GraphPad Prism.

VEGFR1	**Max. % Inhibition**	**EC50 (g/mL)**	**EC50 (pM)**
Avastin	91.9	3.24E-07	2,158
DMS1576	93.8	1.51E-08	201

DMS30000	86.7	3.51E-09	47
DMS30003	89.1	4.42E-09	59
DMS30004	87.1	3.80E-09	51
DMS30005	82.2	2.07E-07	2,763

Table 6B: EC_{50} values of anti-VEGF compounds compared to Bevacizumab (Avastin) in VEGFR2 Receptor Binding Assay.

Curve fitting and EC_{50} calculations were performed using GraphPad Prism.

VEGFR2	Max. % Inhibition	EC50 (g/mL)	EC50 (pM)
Avastin	94.6	6.29E-07	4,192
DMS1576	92.9	1.80E-08	240
DMS30000	94.5	2.98E-09	40
DMS30003	97.9	2.96E-09	40
DMS30004	91.0	3.12E-09	42
DMS30005	85.8	9.55E-08	1,273

Example 18 - Human Umbilical Cord Endothelial Cell (HUVEC) Proliferation assay: inhibition with anti-VEGF dAb-Fc and Fc-dAb molecules

The ability of the Vk dAb-Fc and Fc-dAb molecules to suppress the VEGF driven proliferation of human umbilical vein endothelial cells were compared to that of inhibition in this assay from the Vh dAb-Fc (DMS1576) and Bevacizumab (Avastin) as described in **Example 8**. The Vk dAb-Fc molecules (DMS30005 and DMS30006) were assayed in a single assay, (data not shown).The data indicated that these molecules were less potent (lower IC50) and less able to fully inhibit proliferation, ~70-80%, DMS30005, cf. ~100% for Bevacizumab (Avastin). As previously seen in **Example 8**, the data suggest that the Vh dAb-Fc molecule (DMS1576) has a higher IC50 (i.e. is less potent) than Bevacizumab (Avastin). The Vk Fc-dAb molecules (DMS30000, DMS30003 & DMS30004) were assayed on several occasions. The data sets indicate that the inhibition achieved by treatment with the Vk Fc-dAb molecules gave levels of VEGF-mediated inhibition that matched that achieved with Bevacizumab (Avastin), i.e ~100%. In fact, all Vk Fc-dAb molecules produced best fit curves that overlayed, or were slightly shifted to the left of the similar Bevacizumab, (Avastin) curve so were potentially more potent molecules in this assay, (data not shown).

Example 19 – Demonstration of differential and non-interference of binding of anti-VEGF Vh dAb-Fc and Vk Fc-dAb molecules to human VEGF

The ability of the Vh based dAbFc molecules to bind to VEGF already pre-saturated with Vk based Fc-dAb molecules and, conversely, the ability of Vk based Fc-dAb molecules to bind to VEGF already pre-saturated with Vh based dAbFc molecules was demonstrated in a modified MSD assay using DMS30000 as the Vk based Fc-dAb molecule and DMS1576 as the Vh based dAb-Fc molecule.

19.A – Binding of Vh dAb-Fc DMS1576 to VEGF after pre-saturation with Vk Fc-dAb DMS30000

A MSD high bind 96 well plate (MSD L11XB-3) was coated with 3µg/mL rhVEGF$_{165}$ (sourced 'in house' from transient transfection of HEK293 cells) in PBS (25µl/well), covered with a plate sealer and incubated overnight at 4°C. The next day the MSD plate was washed 3x 300µl/well with Tris wash buffer and blotted over a pad of tissue to remove excess wash buffer from the wells. The MSD plate was then blocked with 3% BSA in PBS (250µl/well) and incubated shaking (750 RPM) at room temperature for 1 hour. After washing the MSD plate, saturating DMS30000 concentrations (≥3µg/ml) were added (25µl/well) and incubated shaking (750 RPM) at room temperature for 1 hour. The MSD plate was washed again before the addition of DMS1576 (25µl/well, 0-100ng/ml) and incubated shaking (750 RPM) at room temperature for 1 hour. The DMS30000 and DMS1576 were prepared in 0.1% BSA in PBS. The MSD plate was washed again before the addition of a detection reagent specific for the Vh dAb contained in DMS1576 (25µL/well, in-house sulfo-TAG labelled anti-Vh mAb) at 1µg/ml in 1% BSA in PBS and incubated with shaking (750 RPM) at room temperature for 1 hour. Prior to measurement of the electrochemiluminescence in a MSD Sector Imager 6000, the MSD plate was washed and 150µL/well of 2x Read Buffer T (MSD R92TC-1) was added.

The data in **Figure 2**A shows that it is possible for the Vh based dAb-Fc molecule DMS1576 to bind VEGF$_{165}$ which has been pre-saturated with Vk based Fc-dAb molecule DMS30000.

19.B – Binding of Vk Fc-dAb DMS30000 to VEGF after pre-saturation with Vh dAb-Fc DMS1576

A MSD high bind 96 well plate (MSD L11XB-3) was coated with 3µg/ml VEGF (sourced 'in house' from transient transfection of HEK293 cells) in PBS (25µl/well), covered with a plate sealer and incubated overnight at 4°C. The next day the MSD plate was washed 3x 300µl/well with Tris wash buffer and blotted over a pad of tissue to remove excess wash buffer from the wells. The MSD plate was then blocked with 3% BSA in PBS (250µl/well) and incubated with shaking (750 RPM) at room temperature for 1 hour. After washing the MSD plate, saturating DMS1576 concentrations (≥3µg/mL) were added (25µL/well) and incubated shaking (750 RPM) at room temperature for 1 hour. The MSD plate was washed again before the addition of DMS30000 (25µL/well, 0-100ng/mL) and incubated shaking (750 RPM) at room temperature for 1 hour. The DMS30000 and DMS1576 were prepared in 0.1% BSA in PBS. The MSD plate was washed again before the addition of a detection reagent specific for the Vk dAb contained in DMS30000 (25µL/well, in-house sulfo-TAG labelled anti-Vk mAb) at 1µg/ml in 1% BSA in PBS and incubated with shaking (750 RPM) at room temperature for 1 hour. Prior to measurement of the electrochemiluminescence in a MSD Sector Imager 6000, the MSD plate was washed and 150µL/well of 2x Read Buffer T (MSD R92TC-1) was added.

The data in **Figure 2B** demonstrates that it is possible for the Vk based Fc-dAb molecule DMS30000 to bind $VEGF_{165}$ which has been pre-saturated with Vh based dAb-Fc molecule DMS1576.

Both sets of data in **Example 19** show that it is possible for both the Vk Fc-dAb (DMS30000) and Vh dAb-Fc, (DMS1576) to bind $VEGF_{165}$ in the presence of the other molecule. The experiments described in **Example 19** suggest that the two lineages of dAb, Vk and Vh derived, may bind to different epitopes on the VEGF homodimer.

Example 20 – Cloning of anti-VEGF dAb-Fc-dAb molecules

The Vh-Vk dAb-Fc-dAbs (SEQ ID NO: 10-11, 25, 38-39 & 53) were engineered by cloning the Vk dAb sequences (DT02-K-044-085 (SEQ ID NO: 97 & 105) or DT02-K-044-251 (SEQ ID NO: 100 & 108) onto the C-terminus of the Vh dAb-Fc (DMS1576, SEQ ID NO:2 & 30) in a mammalian expression vector. The C-terminal dAbs were linked to the C-terminus of Fc using a either the ((GS(TVAAPSGS)x3) (SEQ ID NO:66 & 85), or Albumin Domain 3 (SEQ ID NO:71 & 90) linker sequence. The Vk-Vk dAb-Fc-dAbs (SEQ ID NO:12-15, 26-28, 40-43 & 54-56) were engineered by cloning the Vk dAb sequences (DT02-K-044-085 (SEQ ID NO: 97 & 105) or DT02-K-044-251 (SEQ ID NO:100 & 108) onto the C-terminus of the corresponding Vk dAb-Fc (i.e. either DMS30000 (SEQ ID NO:3 & 31) or DMS30003 (SEQ ID NO:6 & 34) or DMS30013 (SEQ ID NO:16 & 44)) in a mammalian expression vector. The N-terminal dAbs were linked to the N-terminus of Fc using either the AS (SEQ ID NO:58 & 77), or Hinge IgG1 (SEQ ID NO:60 & 79). Site directed mutagenesis was carried out within the Fc region with the following changes for example His 2 Ala or Thr 3 Pro to produce SEQ ID NO: 27 and 28 respectively.

Example 21 – Expression of anti-VEGF dAb-Fc-dAb molecules

Expression plasmids encoding the relevant anti-VEGF dAb-Fc-dAb molecules (listed in SEQ ID NO:10-15, 25-28, 38-43 & 53-56) were transiently transfected into HEK293 6E cells and expressed at 500ml scale to produce the antibody fragment molecules using the method described in **Example 11**. Expression levels of 50-100mg/L supernatant were routinely achieved.

Example 22 – Purification of anti-VEGF dAb-Fc-dAb molecules

The dAb-Fc-dAb molecules were affinity purified from the supernatants (**Example 21**). 2ml of suspended affinity resin in phosphate buffered saline (50:50 slurry) was added to 500ml of filtered supernatant; the supernatant/affinity resin mix was rolled gently at +4°c overnight to allow binding to take place. After which time, the resin was allowed to settle and the supernatant carefully poured off. The resin was re-suspended in

remaining supernatant and poured into an empty drip column and the supernatant was allowed to pass out of the column. The bound product was washed using a combination of pH neutral aqueous buffers to remove non-specifically bound impurities followed by a low pH elution. Over 90% of bound product was recovered and the pH of the elution pool then adjusted to pH 3.5 for 30 minutes to achieve virus neutralisation after which time the pH was adjusted to pH4.5.

Example 23 – Molecular Analysis by Size-Exclusion Chromatography (SEC) of anti-VEGF dAb-Fc-dAb molecules

The molecular integrity, homogeneity and % purity of the anti-VEGF dAb-Fc-dAb molecules which had been purified as described in **Example 22** were analysed by SDS-PAGE and analytical size-exclusion chromatography (SEC) as described in **Example 3.** The proteins were confirmed to be >95% pure target protein by SDS-PAGE and SEC prior to further analysis in biology assays.

Example 24 – VEGF Binding ELISA of anti-VEGF dAb-Fc-dAb molecules

The ability of the anti-VEGF dAb-Fc-dAb molecules to bind specifically to $VEGF_{165}$ was determined by ELISA as described in **Examples 4 & 15**. All of the anti-VEGF dAb-Fc-dAbs tested were found to bind specifically to $VEGF_{165}$ (data not shown).

Example 25 – Binding of anti-VEGF dAb-Fc-dAb molecules to VEGF on Biacore

The binding affinity of the anti-VEGF dAb-Fc-dAb molecules for $VEGF_{165}$ was determined by Surface Plasmon resonance (SPR) using a Biacore T100 in a similar manner to **Example 6**, but with minor modifications. Protein A was immobilised on a C1 chip by primary amine coupling and this surface was then used to capture the anti-VEGF constructs. Human recombinant $VEGF_{165}$ (sourced 'in house' from transient transfection of HEK293 cells) was used as the analyte at 75nM, 15nM, 3nM and 0.6nM. All binding curves were double referenced with a buffer injection (i.e. 0nM) and the data was fitted to 1:1 model inherent to the T100. Regeneration was carried out using 50mM NaOH. The run was carried out at 37°C, using HBS-EP as the running buffer.

The dAb-Fc-dAb molecules were compared to their corresponding Vk Fc-dAb and Vh dAb-Fc molecules. The data for this assay format may suggest that the dAb-Fc-dAb molecules do not appear to be better than the corresponding Vk Fc-dAb. The Vk Fc-dAbs appear to have superior off-rates cf corresponding dAb-Fc-dAbs. However, the traces, (data not shown), may not tell the full story since the dAb-Fc-dAb "affinities" are a mix of two different binding events to VEGF, i.e. the binding of the N-terminal dAbs and the C-terminal Vk dAbs. It is to be expected that the Vk dAb in the dAb-Fc-dAb, i.e. in the same orientation as in the C-terminal Vk Fc-

dAb, will have the same affinity, and this data confirms that previously described in **Example 16.** Positioning of either the Vh or Vk dAb at the N-terminal of the Fc leads to a poorer affinity (see **Example 16**), therefore making the overall affinity of the combined molecule appear worse. The apparent affinities (see **Table 7**) show the following potency on the Biacore for these molecules: the Vh-Vk dAb-Fc-dAbs (DMS30007 and DMS30008) have the poorest affinitys, the Vk-Vk dAb-Fc-dAbs (DMS30009, DMS30010, DMS30011 and DMS30012) have the best of the dAb-Fc-dAb affinities and appear to be very similar to one another.

Table 7: Binding of anti-VEGF dAb-Fc-dAb molecules and corresponding dAb-Fc- and Fc-dAb molecules to $VEGF_{165}$

Construct	Model	ka	kd	KD(pM)
DMS1576	1:1 Binding	3.04E+06	4.24E-04	139.5
DMS30000	1:1 Binding	2.28E+07	1.60E-05	0.7
DMS30007	1:1 Binding	2.82E+06	1.22E-04	43.3
DMS30009	1:1 Binding	2.96E+07	6.92E-05	2.3
DMS30010	1:1 Binding	2.64E+07	8.08E-05	3.1
DMS30003	1:1 Binding	1.81E+07	1.37E-05	0.8
DMS30008	1:1 Binding	2.56E+06	1.15E-04	44.9
DMS30011	1:1 Binding	1.77E+07	8.07E-05	4.6
DMS30012	1:1 Binding	1.61E+07	7.39E-05	4.6

Example 26 – VEGF R1 & R2 Receptor Binding Assay of anti-VEGF dAb-Fc-dAb molecules

The potencies of the anti-VEGF Vh-Vk and Vk-Vk dAb-Fc-dAb molecules were analysed in the VEGF receptor, R1 and R2, binding assay. The potencies (EC50) against both VEGFR1 and VEGFR2 of the dAb-Fc-dAbs was seen to match that of the Vk Fc-dAb molecule (DMS30000) and seen to be more potent (i.e. lower EC50 values) than both the Vh dAb-Fc molecule (DMS1576) and Bevacizumab (Avastin), see **Table 8 A and B**. Against VEGFR1, the data indicate that the inhibition achieved by both Vh-Vk dAb-Fc-dAbs (DMS30007 and DMS30008) and the Vk-Vk dAb-Fc-dAb (DMS30009) matched that achieved with the Vh dAb-Fc molecule (DMS1576), the Vk Fc-dAb molecule (DMS30000) and Bevacizumab (Avastin) all ≥90%, **(Table 8A)**. Against VEGFR2, the data indicate that the inhibition achieved by the Vh-Vk (DMS30022) dAb-Fc-dAb matched that achieved with the Vh dAb-Fc molecule (DMS1576), the Vk Fc-dAb molecules (DMS30000 and DMS30003) and Bevacizumab (Avastin), all achieved ≥90%; whereas the inhibition achieved by Vh-Vk dAb-Fc-dAbs (DMS30007 and DMS30008) and the Vk-Vk dAb-Fc-dAb molecules (DMS30009, DMS30023 and DMS30025) was lower at ~75-87%, (**Table 8B**).

Table 8A: EC_{50} values of anti-VEGF dAb-Fc-dAb molecules compared to Bevacizumab (Avastin) in VEGFR1 Receptor Binding Assay.

Curve fitting and EC_{50} calculations were performed using GraphPad Prism.

VEGFR1	Max. % Inhibition	EC50 (g/mL)	EC50 (pM)
Avastin	92.0	3.45E-07	2,297
DMS1576	95.8	2.15E-08	287
DMS30000	89.5	5.09E-09	68
DMS30003	92.0	5.94E-09	79
DMS30007	92.8	5.90E-09	59
DMS30008	92.0	6.00E-09	60
DMS30009	92.6	4.17E-09	42

Table 8B: EC_{50} values of anti-VEGF dAb-Fc-dAb molecules compared to Bevacizumab, (Avastin), in VEGFR2 Receptor Binding Assay.

(i) Curve fitting and EC_{50} calculations were performed using GraphPad Prism.

VEGFR2	Max. % Inhibition	EC50 (g/mL)	EC50 (pM)
Avastin	94.3	5.52E-07	3,679
DMS1576	96.7	3.30E-08	439
DMS30000	93.6	5.01E-09	67
DMS30003	98.3	4.56E-09	61
DMS30007	81.3	8.02E-09	80
DMS30008	83.0	7.20E-09	72
DMS30009	86.6	5.52E-09	55

(ii)

VEGFR2 RBA	Max. % Inhibition	EC50 (g/mL)	EC50 (pM)
Avastin	93.6	4.67E-07	3113
DMS1576	95.9	6.72E-08	896
DMS30000 (Batch 1)	89.8	4.22E-09	56
DMS30000 (Batch 2)	97.7	3.30E-09	44

DMS30022	95.0	8.17E-09	82
DMS30023	74.7	5.20E-09	52
DMS30025	82.4	4.86E-09	49

Example 27 - Human Umbilical Cord Endothelial Cell (HUVEC) Proliferation assay of anti-VEGF dAb-Fc-dAb molecules

The abilities of the Vh-Vk (DMS30022) and Vk-Vk (DMS30023 and DMS30025) dAb-Fc-dAb molecules to suppress proliferation of human umbilical vein endothelial cells were compared to the Vh dAb-Fc molecule (DMS1576), the Vk Fc-dAb molecule (DMS30000) and Bevacizumab (Avastin) using the method described in **Examples 8 & 18** with the following deviations (i) rather than leaving the outer wells free of cells, the whole 96 well plate was used and (ii) the data was analysed using GraphPad Prism using a Sigmodial curve fit, variable slope cf a non-linear regression (variable slope). The data suggest that, as previously seen in **Examples 8 and 18**, the Vh dAb-Fc molecule (DMS1576) has a higher EC50 (i.e. is less potent) than Bevacizumab (Avastin); on average, it was seen that the Vk Fc-dAb molecule (DMS30000) was similar in terms of EC50 to Bevacizumab (Avastin); whereas all the Vh-Vk and Vk-Vk dAb-Fc-dAb molecules (DMS300022, DMS300023, DMS300024 and DMS300025) were all seen to be improved over Bevacizumab (Avastin) in terms of EC50.

Example 28 – Performance of dAb-Fc-dAb and dAb-Fc and Fc-dAb formats in VEGF binding assays measured in solution by Isothermal Calorimetry (ITC)

The solution equilibrium binding affinity (K_D), stoichiometry (N) and thermodynamics (ΔH, enthalpy and ΔS, entropy) of the anti-VEGF dAb-Fc-dAbs, dAb-Fcs and Fc-dAbs binding to $VEGF_{165}$ was determined by isothermal titration calorimetry (ITC) using a Microcal VP-ITC and compared to that of the monoclonal antibody Bevacizumab (Avastin). The main aim of the experiment was to compare the relative stoichiometry of binding to VEGF of the different molecular formats. $VEGF_{165}$ was titrated into antibody solutions at 25°C until there was greater than 3 fold concentration excess of $VEGF_{165}$ and saturation was achieved. All titrations used the same batch of $VEGF_{165}$ to ensure consistency. The integrated binding isotherms were fitted within the Origin software (Microcal version) using a standard 1:1 binding model as there were no signs of multiphasic behavior. The results are summarised in **Table 10.**

Table 10: Binding of anti-VEGF dAb-Fc-dAb molecules, dAb-Fcs and Fc-dAb molecules to $VEGF_{165}$

Construct	**N** (VEGF165(mono	**K_A M^{-1}**	**ΔH** (cal/mol)	**ΔS** (cal/mol/deg)

	mer)/antibody)			
Avastin	1.5	4.96E+07	-1.59E+04	-18.1
DMS30000 (Fc-dAb)	1.2	8.00E+07	-1.95E+04	-29.3
DMS30023 (dAb-Fc-dAb)	2.1	3.78E+07	-1.93E+04	-29.9
DMS30022 (dAb-Fc-dAb)	1.9	1.08E+08	-2.20E+04	-37.0
DMS30028 (dAb-Fc)	1.3	1.45E+07	-1.73E+04	-25.4
DMS51576 (dAb-Fc)	0.95	1.18E+08	-2.08E+04	-32.9
Avastin	1.5	5.36E+07	-1.82E+04	-25.6

All antibodies have enthalpically favourable and entropically unfavourable binding at 25°C. There is a clear distinction in the stoichiometry between the dAb-Fc, Fc-dAb and dAb-Fc-dAb dAb formats, with a larger capacity on for the dual dAb formats. The dAb-Fc-dAb formats also show a higher capacity than Avastin when $VEGF_{165}$ is present in excess. The affinities measured are in same rank order and consistent with those measured by other methods.

Example 29 – Cloning of anti-VEGF Vh dAb-Fc, Vk dAb-Fc and Vk Fc-dAb Molecules with Various Linker Modifications The dAb sequences (SEQ ID NO: 96-97, 104-105, see **Table 19**) were cloned onto the N- or C-terminus of a generic Fc of the human IgG1 isotype in a mammalian expression vector. The dAbs were linked to the Fc using a variety of linker sequences (see **Table 19**, SEQ ID Nos 57-75 & 76-94): For the Vh dAb-Fc the dAb was DOM15-26-597; the N-terminal linkers were either AS (SEQ ID NO:58 & 77), or TVAAPS (SEQ ID NO:59 & 78), or Hinge IgG1 linker (SEQ ID NO:60 & 79), or Hinge IgG3 linker (SEQ ID NO:61 & 80) or Fibronectin x3 linker (SEQ ID NO:62 & 81) or Fibronectin x4 linker (SEQ ID NO:63 & 82); For the Vk dAb-Fc the dAb was DT02-K-044-085; the N-terminal linkers were either AS with a H2A IgG1Fc point mutation (SEQ ID NO:64 & 83), AS with a T3P IgG1Fc point mutation (SEQ ID NO:65 & 84), or TVAAPS (SEQ ID NO:59 & 78), or Hinge IgG1 linker (SEQ ID NO:60 & 79), or Hinge IgG3 linker (SEQ ID NO:61 & 80) or Fibronectin x3 linker (SEQ ID NO:62 & 81) or Fibronectin x4 linker (SEQ ID NO:63 & 82); and the Vk Fc-dAb was DT02-K-044-085; the C-terminal linkers were either (GS(TVAAPSGS)x3 (SEQ ID NO:66 & 85), or Fibronectin x3 linker (SEQ ID NO:67 & 86) or Fibronectin x4 linker (SEQ ID NO:68 & 87) or Albumin Domain 1 (SEQ ID NO:69 & 88), Albumin Domain 2 (SEQ ID NO:70 & 89), Truncated Albumin Domain 3 linker, (Alb Dom 3-TFHAD, SEQ ID NO:72 & 91) or Gly4Ser 3x Linker, (SEQ ID NO:73 & 92), Gly4Ser 4x Linker, (SEQ ID NO:74 & 93) or Helical Linker, (SEQ ID NO:75 & 94) .

Example 30 – Expression of anti-VEGF Vh dAb-Fc, Vk dAb-Fc and Vk Fc-dAb Molecules with Various Linker Modifications

Expression plasmids encoding the relevant Vh anti-VEGF dAb-Fc, Vk anti-VEGF dAb-Fc and Vk anti-VEGF Fc-dAb molecules (described in **Example 29** SEQ ID NO: 132-134 and 155-176, Table 19) transiently transfected into HEK293 6E cells and expressed at 500ml scale to produce the antibody fragment molecules, as described in **Example 11.**

Example 31 – Purification of of anti-VEGF Vh dAb-Fc, Vk dAb-Fc and Vk Fc-dAb Molecules with Various Linker Modifications

The Vh anti-VEGF dAb-Fc, Vk anti-VEGF dAb-Fc and Vk anti-VEGF Fc-dAb molecules described in **Example 30** were affinity purified from the supernatants (see **Example 12**).

Example 32 – Molecular Analysis by SDS-PAGE and Size-Exclusion Chromatography (SEC) of anti-VEGF Vh dAb-Fc, Vk dAb-Fc and Fc-dAb Molecules with Various Linker Modifications

The molecular integrity, homogeneity and % purity of the anti-VEGF Vh dAb-Fc, anti-VEGF Vk dAb-Fc and anti-VEGF Vk Fc-dAb molecules which had been purified as described in **Example 31** were analysed by SDS-PAGE, as described in **Example 3**, The gels showed band sizes consistent with the predicted molecular mass of the intact mature protein (from ~76kDa to ~85kDa for dAb-Fc and Fc-dAb molecules), allowing for the presence of the predicted glycan chains per monomer chain. The proteins were confirmed to be >95% pure target protein by SDS-PAGE and SEC prior to further analysis in biology assays. If the dAb-Fc or Fc-dAb molecule was <95% pure a further SEC purification was carried out (see **Example 14 & 33**).

Example 33 – Purification by Size-Exclusion Chromatography (SEC) of of anti-VEGF Vh dAb-Fc, Vk dAb-Fc and Vk Fc-dAb Molecules with Various Linker Modifications

If necessary, preparative size-exclusion chromatography (SEC) was carried out for the Vh dAb-Fc, Vk dAb-Fc and Vk Fc-dAb molecules.

Example 34 – Binding of anti-VEGF Vh dAb-Fc, Vk dAb-Fc and Fc-dAb Molecules with Various Linker Modifications to VEGF on Biacore

The binding affinity of the anti-VEGF Vh dAb-Fc, Vk dAb-Fc and Fc-dAb molecules for $VEGF_{165}$ was determined by Surface Plasmon resonance (SPR) using a Biacore T100 in a similar manner to **Example 16**, but with minor modifications. Protein A was immobilised on a C1 chip by primary amine coupling and this surface was then

used to capture the anti-VEGF constructs. Human recombinant $VEGF_{165}$ (sourced 'in house' from transient transfection of HEK293 cells) was used as the analyte over a varying range of diliution series detailed below. All binding curves were double referenced with a buffer injection (i.e. 0nM) and the data was fitted to 1:1 model inherent to the T100. Regeneration was carried out using 50mM NaOH. The run was carried out at 37°C, using HBS-EP as the running buffer.

The Vk Fc-dAb molecules were compared to DMS30000 and Bevacizumab (Avastin) over a concentration range of 16nM, 4nM, 2nM, 1nM, 0.5nM and 0.25nM. From the data it was concluded that the Vk Fc-dAb molecules with the DT02-K-085 dAb linked to the IgG1Fc by C-terminal linkers Albumin domain 2 and Fibronectin x4 behaved similarly in th Biacore to DMS30000, (Table 11A). Use of the albumin 2 linker was not preferred from biophysical studies, (data not shown), so the Fibronectin 4 (fib4) was the preferred C-terminal linker to replace the (GS(TVAAPSGS)x3 linker in DMS3000, the latter linker contributing many glycosylated isoforms making molecule development problematic, (data not shown). A further anti-VEGF VkFc-dAb data set was generated by similar Biacore experiments, over a concentration range of 128nM to 0.03125nM in a 4 fold dilution series, and is shown in Table 11B. The data compares the fib4 linker (DMS30026) with helical linker (DMS30027) as the C-terminal to attach the DT02-K-085 dAb to the IgG1Fc. The fib4 linker is preferred.

Table 11A: Binding of anti-VEGF Vk dAb-Fc and Fc-dAb molecules to $VEGF_{165}$

and comparison to DMS1576, DMS30000, anti-VEGF dAb-Fc-dAbs and Bevacizumab (Avastin)

sample	Ka (M-1.s-1)	Kd (s-1)	KD (M)	fit
C085 Alb DOM1	4.15E+06	1.12E-04	2.70E-11	ok
C085 Alb DOM2	7.52E+06	Too tight	na	ok
C085 Alb DOM3	6.17E+06	8.12E-05	1.32E-11	ok
C085 Fibrx4	7.72E+06	At the limit of measurement	na	ok
C085 G4Sx3	6.19E+06	1.80E-04	2.91E-11	ok
C085 G4Sx4	6.11E+06	1.41E-04	2.31E-11	ok
DMS1576	3.48E+06	3.05E-04	8.77E-11	Not ideal/ doesn't look like 1:1 interaction
DMS30000	8.96E+06	Too tight	na	ok
DMS30013	8.74E+06	Too tight	na	ok
DMS30014	8.68E+06	At the limit of measurement	na	ok
N085 Fibrx4	6.76E+06	4.83E-05	7.15E-12	Not ideal/ doesn't look like 1:1 interaction
N085 TVAAPS	8.07E+06	Too tight	na	Not ideal/ doesn't look like 1:1 interaction
C085 Alb DOM1	4.15E+06	1.12E-04	2.70E-11	ok
avastin	run	failed	na	bad

Table 11B: Binding of anti-VEGF Vh dAb-Fc, Vk dAb-Fc and Vk Fc-dAb molecules to $VEGF_{165}$ and comparison to DMS1576 and Bevacizumab (Avastin)

Sample	Ka (M-1.s-1)	Kd (s-1)	KD (M)	comment
Avastin	3.18E+05	na	na	Off-rate value outside the range and thus not reported
C085 helical	1.00E+07	6.23E-05	6.21E-12	Good fit to the 1:1binding model
N085 extg1	1.03E+07	6.60E-05	6.43E-12	Biphasic curves
C085 Fibr4	8.12E+06	1.29E-05	1.59E-12	Good fit to the 1:1binding model Off-rate value approching the limit of measurement: caution!
N597 Fibr3	1.88E+06	1.31E-05	6.96E-12	Off-rate value approching the limit of measurement: caution!
N085 T2P	9.40E+06	7.57E-05	8.06E-12	Biphasic curves
DMS1576	1.60E+06	2.09E-04	1.30E-10	Biphasic curves

The Vk dAb-Fc molecules were compared to DMS1576 and Bevacizumab (Avastin) over a concentration range of 16nM, 4nM, 2nM, 1nM, 0.5nM and 0.25nMm, where Vk dAbFc molecules with the DT02-K-085 dAb linked to the IgG1Fc by N-terminal linkers N085 Fibronectin 4 and 1xTVAAAPS were compared. The data suggests that the 1xTVAPPS is the preferred of the two linkers to attach the DT02-K-085 dAb to the N-terminus of the IgG1Fc. Further examples of Vk dAb-Fc molecules are shown in Tables 11B and 11C. The data was generated over a concentration range of 128nM to 0.03125nM in a 4 fold dilution series and the Vk dAb-Fc proteins with the DT02-K-085 dAb attached to the N-terminus of the IgG1Fc by N085 T113P (T2P) or ASTHP linker (DMS30029), N085 extg1 or IgG1 Hinge linker (DMS30028) compared to DMS1576. From the data in Table 11B, the IgG1 Hinge linker, (N085extg1), was taken as the preferred linker for the DT02-K-085 dAb attachment to the N-terminus of the IgG1Fc, ahead of the 1x TVAAPS.

The Vh dAb-Fc molecules were compared to DMS1576 and Bevacizumab (Avastin) over a concentration range of 128nM to 0.03125nM in a 4 fold dilution series. Data from Table 11B suggest that the preferred linker for attachment of the 15-26-597 Vh dAb to the N-terminus of the dAb-Fc is that based upon 3 copies of the Fibronectin repeat. Further data, shown in Table 11C suggest that when other linkers are used to N-terminally attach the 15-26-597 dAb to the Fc, the resultant dAb-Fc proteins generated are biphasic nature in anti VEGF Biacore. From the shape of the Biacore curves the N597extg1 (IgG1 Hinge) linker was taken to be the next best from this data set.

Table 11C: Binding of anti-VEGF VhdAb-Fc, Vk dAb-Fc molecules to $VEGF_{165}$ and comparison to DMS1576 and Bevacizumab (Avastin)

Sample	Ka (M-1 s-1)	Kd (s-1)	KD (M)	comment
Avastin	3.24E+05	na	na	Kd is out of the range (run at 60ul/min)
Avastin new	2.85E+05	1.92E-05	6.74E-11	Kd is approching the limit of detection. Caution!
Avastin new	3.14E+05	na	na	Kd is out of the range (run at 60ul/min)
DMS1576	7.57E+05	2.10E-04	2.77E-10	Biphasic curves. Doesn't look like a 1:1 binding
N085 TVAAPS	1.16E+07	9.08E-05	7.86E-12	Biphasic curves. Doesn't look like a 1:1 binding Molecule better DMS1576than mainly due to a better on-rate
N597 extg1	1.22E+06	1.79E-04	1.47E-10	Biphasic curves. Doesn't look like a 1:1 binding Molecule quite similar to DMS1576
N597 extg3	1.33E+06	1.70E-04	1.28E-10	Biphasic curves. Doesn't look like a 1:1 binding Molecule quite similar to DMS1576

The behaviour in anti-VEGF Biacore of the Vh dAb-Fc molecules containing the linkers Fib3 or Fib4 for the N-terminal coupling of the 15-26-597 dAb to the Fc with DMS1576 were compared over a concentration range of VEGF 32nM to 0.03125nM in a 4 fold dilution series. The data is shown in **Table 11D.** The data suggest that there is no advantage to using the Fib4 linker over the Fib3 linker but that when the linker is lengthened from the 'AS' present in DMS1576 for the N-terminal attachment of the 15-26-597 dAb to the Fc with the sequences of the Fib3 or Fib4 linker the Vh dAb-Fc shows more optimal binding kinetics by anti VEGF Biacore.

The overall data set suggested that to build the most optimal anti-VEGF dAb-Fc-dAb using the novel linkers to improve potency but also reduce the number of glycosylated isoforms then for:

(i) the C-terminal addition of the DT-02-K44-085 Vk dAb to the Fc; the Fibronectin x4 and helical linkers were preferred;

(ii) the N-terminal addition of the DT-02-K44-085 Vk dAb to the Fc; the IgG1 Hinge, ASTHP and 1xTVAAPs linkers were preferred;

(iii) the N-terminal addition of the 15-26-597 Vh dAb to the Fc; the Fibronectin 3 linker was preferred, but the IgG1 Hinge and AS linkers were additionally pursued

Table 11D: Binding of anti-VEGF VhdAb-Fc molecules with Fibr4 and Fibr3 N-terminal linkers to $VEGF_{165}$ and comparison to DMS1576.

Ligand	Ka (M-1 s-1)	Kd (s-1)	KD (M)	comments
N597 Fibr4	2.20E+06	7.09E-05	3.19E-11	Good fit
N597 Fibr3	2.13E+06	7.83E-05	3.67E-11	Good fit
DMS1576	1.87E+06	1.77E-04	9.43E-11	Decent fit

Example 35 – Cloning of anti-VEGF Vh/Vk & Vk/Vk dAb-Fc-dAb molecules with modified linkers

The Vk-Vk dAb-Fc-dAbs: DMS30034 and DMS30035 (SEQ ID NO: 135-136, 177-178, Table 19) and the Vh-Vk dAb-Fc-dAbs: DMS30036-DMS30041 (SEQ ID NO: 137-142, 179-184, Table 19 were engineered by cloning the Vk dAb sequence (DT02-K-044-085 (SEQ ID NO: 97 & 105) or the Vh dAb sequence, DOM15-26-597 (SEQ ID: 96 & 104) from the Vh-dAb-Fc fusion vector (DMS1576, SEQ ID NO: 2 & 30) onto the N-terminus or C-terminus of the Fc IgG1 sequence (SEQ ID: 102 & 110). The Fc IgG1 was linked at the C-terminus to the N-terminus of the Vk dAb (DT02-K-044-085 (SEQ ID NO: 97 & 105)) by a Fibronectin x4 linker (SEQ ID: 63 & 82, see Table 19) in a mammalian expression vector. The N-terminal Vh dAbs were linked to the N-terminus of Fc using either the AS (SEQ ID NO:58 & 77, Table 19): DMS30038 (SEQ ID NO:139, 181, Table 19), or Hinge IgG1 (SEQ ID NO:60 & 79, Table 19): DMS30037 (SEQ ID NO:138, 180, Table 19) or Fibronectin 3 (SEQ ID NO:67 & 86, Table 19): DMS30036 (SEQ ID NO:137, 179, Table 19) and DMS30040 (SEQ ID NO:141, 183, Table 19) or Fibronectin 4 (SEQ ID NO:60 & 79, Table 19): DMS30039 (SEQ ID NO:140, 182, Table 19) and DMS30041 (SEQ ID NO:142, 184, Table 19 -). The N-terminal Vk dAbs were linked to the N-terminus of Fc using either the Hinge IgG1 (SEQ ID NO:60 & 79, Table 19): DMS30034 (SEQ ID NO:135, 177, Table 19) or ASTHP, (H2A IgG1Fc, SEQ ID NO: 58 & 65; 77 & 84, Table 19): DMS30035 (SEQ ID NO:136, 178, Table 19) or Hinge IgG1 (SEQ ID NO:60 & 79, Table 19-): DMS30043 (SEQ ID NO: 143, 177, Table 19). In sequences DMS 30035, 30040 and 30041 (SEQ IDs 136 & 178; 141 & 183; and 142 & 148 respectively) the Fc contained proline instead of threonine at position 3 of the Fc.

The fibronectin x 4 linker sequence and C-terminal Vk dAb were codon optimised to raise the overall dAb-Fc-dAb codon adaptation index (human) to >0.95, for stable expression in mammalian cells. This fragment was generated by oligonucleotide assembly and cloned into DMS30034 (SEQ ID 135 & 177) , DMS30036 (SEQ ID 137 & 179), DMS30037, (SEQ ID 138 & 180) and DMS30038 (SEQ ID 139 & 181) to generate DMS30043 (SEQ ID 143 & 177) , DMS30044 (SEQ ID 144 & 179), DMS30045 (SEQ ID 145 & 180) and DMS30046 (SEQ ID 146 & 181), respectively. For clarification, the protein amino acid sequences remained the same only the DNA sequence was altered.

Example 36 – Expression of anti-VEGF Vh/Vk & Vk/Vk dAb-Fc-dAb molecules with modified linkers

Expression plasmids encoding the relevant anti-VEGF dAb-Fc-dAb molecules (listed in SEQ ID NO:135, 137-139 and 177, 179-181) were transiently transfected into HEK293 6E cells and expressed at 500ml scale to produce the antibody fragment molecules using the method described in **Examples 10** and **21**. Expression levels of >30mg/L supernatant were routinely achieved.

Example 37 – Purification of of anti-VEGF Vh/Vk & Vk/Vk dAb-Fc-dAb molecules with modified linkers

The dAb-Fc-dAb molecules were affinity purified from the supernatants (**Example 36**). 2ml of suspended affinity resin in phosphate buffered saline (50:50 slurry) was added to 500ml of filtered supernatant; the supernatant/affinity resin mix was rolled gently at +4°c overnight to allow binding to take place. After which time, the resin was allowed to settle and the supernatant carefully poured off. The resin was re-suspended in remaining supernatant and poured into an empty drip column and the supernatant was allowed to pass out of the column. The bound product was washed using a combination of pH neutral aqueous buffers to remove non-specifically bound impurities followed by a low pH elution. Over 90% of bound product was recovered and the pH of the elution pool then adjusted to pH 3.5 for 30 minutes to achieve virus neutralisation after which time the pH was adjusted to pH 4.5.

Example 38 – Molecular Analysis by Size-Exclusion Chromatography (SEC) of anti-VEGF Vh/Vk & Vk/Vk dAb-Fc-dAb molecules with modified linkers

The molecular integrity, homogeneity and % purity of the anti-VEGF dAb-Fc-dAb molecules which had been purified as described in Example 38 were analysed by SDS-PAGE and analytical size-exclusion chromatography (SEC) as described in **Examples 3, 13 & 24**. The proteins were confirmed to be >95% pure target protein by SDS-PAGE and SEC prior to further analysis in biology assays.

Example 39 – Binding of anti-VEGF Vh/Vk & Vk/Vk dAb-Fc-dAb molecules with modified linkers to VEGF and comparison to Vh and Vk dAb-Fcs and Vk Fc-dAbs on Biacore.

The binding affinity of the anti-VEGF dAb-Fc-dAb molecules for $VEGF_{165}$ was determined by Surface Plasmon resonance (SPR) using a Biacore T100 in a similar manner to **Examples 25 & 34**, but with minor modifications. Protein A was immobilised on a C1 chip by primary amine coupling and this surface was then used to capture the anti-VEGF constructs. Human recombinant $VEGF_{165}$ (sourced 'in house' from transient transfection of HEK293 cells) was used as the analyte at 32nM to 0.03125nM in a 4 fold dilution series. All binding curves were double referenced with a buffer injection (i.e. 0nM) and the data was fitted to 1:1 model inherent to the T100. Regeneration was carried out using 50mM NaOH. The run was carried out at 37°C, using HBS-EP as the running buffer.

The dAb-Fc-dAb molecules were compared to their corresponding Vk Fc-dAb and Vk dAb-Fc and Vh dAb-Fc molecules, the data is shown in Table 12A. Some of these Vh / Vk dAb-Fc-dAbs: DMS30037 and DMS30022, (see Table 12A), performed unexpectedly well in the Biacore and had good on and off rates suggesting highly potent molecules.

Table 12A: Binding of anti-VEGF dAb-Fc-dAb molecules and corresponding dAb-Fc- and Fc-dAb molecules to $VEGF_{165}$

Ligand	Ka (M-1,s-1)	Kd (s-1)	KD (M)
DMS30036	4.01E+06	1.49E-05	3.70E-12
Avastin	5.40E+05	Out of the range	Out of the range
DMS30030	2.45E+06	Out of the range	Out of the range
DMS30037	4.35E+06	1.57E-05	3.61E-12
DMS30034	1.18E+07	8.11E-05	6.90E-12
DMS30026	8.49E+06	Out of the range	Out of the range
DMS30000ITC	1.31E+07	2.29E-05	1.74E-12
DMS30028ITC	1.04E+07	1.07E-04	1.03E-11
DMS30022ITC	6.93E+06	1.27E-05	1.83E-12
DMS30023ITC	1.33E+07	9.13E-05	6.85E-12
DMS1576ITC	2.70E+06	1.96E-04	7.24E-11
AvastinITC	4.60E+05	Out of the range	Out of the range

Further Biacore data sets were obtained comparing the most potent looking dAb-Fc-dAbs with DMS1576 and Avastin and an example of this is shown in Table 12B. The data sets re-affirm that the Vh/Vk dAb-Fc-dAbs: DMS30022, DMS30036, DMS30037 and DMS30038 look similar in potency to Avastin and that the Vk / Vk dAb-Fc-dAb DMS30034 is overall less potent, (in terms of off-rate Kd s-1 1.04E-04, see Table 12B), though an improvement over DMS1576 in overall KD, (see Table 12B).

In contrast to **Example 25** the dAb-Fc-dAb molecule to VEGF binding data which is seen in these results suggests that the Biacore can be a very informative assay format for analysing the bivalent anti-VEGF dAb-Fc-dAb molecules, when both VEGF binding sites are of similar potency (eg. DMS30036, DMS30037 and DMS30038), and can be used to distinguish from molecules that have one potent and one less potent binding site, eg DMS30034.

Table 12B: Binding of anti-VEGF dAb-Fc-dAb molecules molecules to $VEGF_{165}$ and comparison to DMS1576 and Avastin.

Ligand	Ka (M-1 s-1)	Kd (s-1)	KD (M)
DMS30022	1.01E+07	5.10E-05	5.06E-12
Avastin	5.61E+05	Out of the range	Out of the range
DMS30034 HEK	2.57E+07	1.04E-04	4.06E-12
DMS30037 HEK	8.01E+06	4.98E-05	6.22E-12
DMS1576	3.62E+06	2.97E-04	8.21E-11
DMS30036 HEK	6.66E+06	4.85E-05	7.28E-12
DMS30038 HEK	7.11E+06	4.74E-05	6.67E-12

Example 40 – VEGF R2 Receptor Binding Assay of anti-VEGF Vh/Vk & Vk/Vk dAb-Fc-dAb molecules with modified linkers compared to most potent Vh dAb-Fc and Avastin.

The potencies of some of the anti-VEGF Vh/Vk & Vk/Vk dAb-Fc-dAb molecules with modified linkers were analysed in the VEGF receptor 2, (R2), binding assay using the modified method, i.e. with no pre-incubation, described in **Examples 7, 17 & 26** and were compared to the Vh dAb-Fc, DMS30030 and Bevacizumab (Avastin). The data is shown in Tables 13A and 13B. From the data in Table 13A all the tested dAb-Fc-dAb molecules: DMS30022, DMS30023, DMS30034, DMS30036 and DMS30037 appeared to be more potent by lower EC50 values than DMS30030 and considerably more potent than Bevacizumab (Avastin). However, there was some variability in the maximal percentage inhibition achived by the molecules in the assay with Avastin, DMS30022 and DMS30030 achieving >94% maximal inhibition and DMS30023, DMS30034, DMS30036 and DMS30037 achieving 78-84% maximal inhibition, (data not shown)

Further data was generated comparing the dAb-Fc-dAbs: DMS30022 and DMS30038 with the Vh dAb-Fc, DMS30030 and Bevacizumab (Avastin) and this is shown in Table 13B.

In summary, DMS30022 and DMS30038 appeared to be comparable and more potent by lower EC50 values than DMS30030 and considerably more potent than Bevacizumab (Avastin), Table 13B. Again, there was some variability in the maximal percentage inhibition achived by the molecules in the assay with Avastin, DMS30022 and DMS30030 achieving >95% maximal inhibition and DMS30038 achieving 91% maximal inhibition, (data not shown).

Table 13A: EC_{50} values of anti-VEGF dAb-Fc-dAbs compared to DMS30030 and Bevacizumab (Avastin) in VEGFR2 Receptor Binding Assay.

Curve fitting and EC_{50} calculations were performed using GraphPad Prism.

VEGFR2 RBA	EC50 (g/mL)	EC50 (pM)
Avastin	2.92E-07	1944
DMS30034 (LT111020)	4.93E-09	47
DMS30036 (LT111027)	3.78E-09	36
DMS30037 (LT111020)	3.86E-09	37
DMS30022 (CR290911)	4.04E-09	38
DMS30023 (CR290911)	3.23E-09	31
DMS30030 (MH171011)	4.93E-09	62

Table 13B: EC_{50} values of anti-VEGF dAb-Fc-dAbs: DMS30022 and DMS30038 compared to DMS30030 and Bevacizumab (Avastin) in VEGFR2 Receptor Binding Assay.

Curve fitting and EC_{50} calculations were performed using GraphPad Prism.

VEGF R2 RBA	EC50 (g/mL)	EC50 (pM)
Avastin	2.9E-07	1933
DMS30022 (CR290911)	4.67E-09	44
DMS30038 (CR291111)	4.12E-09	39
DMS30030 (MH171011)	1.17E-08	146

Example 41 - Human Umbilical Vein Endothelial Cell (HUVEC) Proliferation assay: inhibition with anti-VEGF Vh/Vk & Vk/Vk dAb-Fc-dAb molecules containing modified linkers

The abilities of the dAb-Fc-dAb molecules with modified linkers: Vh-Vk (DMS30022, DMS30036, DMS30037) and Vk-Vk (DMS30023, DMS30034), to suppress proliferation of human umbilical vein endothelial cells were compared to the Vh dAb-Fc molecule (DMS30030), the Vk Fc-dAb molecule (DMS30026) and Bevacizumab (Avastin) using the method described in **Examples 8 & 18 & 27** with the following deviations (i) rather than leaving the outer wells free of cells, the whole 96 well plate was used and (ii) the data was analysed using GraphPad Prism using a Sigmodial curve fit, variable slope cf a non-linear regression (variable slope). The test compounds were independently assessed on individual plates against the comparator molecule, Bevacizumab (Avastin); the assay was carried out on at least four separate occasions, with a total data set per molecule of Bevacizumab (Avastin): 20; DMS30030: 8; DMS30036: 8; DMS30022: 4; DMS30037: 8; DMS30026: 4; DMS30023: 4; DMS30034: 4 (data not shown). The focus was upon analysing both the degree of maximum inhibition and the relative EC50 values in the assay generated by certain molecules compared to that of Bevacizumab (Avastin).

The data was analysed using GraphPad Prism using a Sigmodial curve fit, variable slope cf a non-linear regression (variable slope). Individual curve fits were fitted for each molecule and at each day. Due to some

poor fitting, it was decided to introduce constraints for the curve where a plateau was not observed at the lower concentration. This constraint would be equal to the mean of the points at the lowest concentration. Data was manually selected as to whether the minimum was constrained or not, and the curve fit and parameters were automatically updated based upon this criteria selection. Estimates of the curve maxima and the standard error were analysed using a weighted mixed model analysis of variance, using 1/ (standard error)^2 , [SE]^2, as a weighting. The analysis adjusted for variability between plates and days using random effects terms. From this analysis, the predicted means were estimated and comparisons were made back to Avastin (control) (**Table 14A**). The same analysis was then performed on the log10 scale for the IC50, and the results back transformed. From this, estimates of the geometric means were generated and comparisons were made back to Avastin in the form of a ratio to Avastin (control) i.e. a ratio of 0.5 would indicate a 50% drop from Avastin (**Table 14B**).

Table 14A: Predicted geometric means of maximum percentage inhibition of anti-VEGF dAb-Fc-dAbs with 95% confidence intervals (CI) compared to DMS30026, DMS30030 and Bevacizumab (Avastin) in the HUVEC Assay.

Predicted Means for Max % Inhibition			
mAb	Estimate	Lower 95% CI	Upper 95% CI
Avastin	73.5423	68.5130	78.5717
DMS30022	80.3745	70.6202	90.1287
DMS30023	69.7258	60.3045	79.1471
DMS30026	70.5232	59.1251	81.9213
DMS30030	64.7441	57.5772	71.9110
DMS30034	85.1436	73.9429	96.3444
DMS30036	79.0377	73.2432	84.8323
DMS30037	77.8069	72.1446	83.4691

From this analysis molecules DMS30022, DMS30034, DMS30036 and DMS30037 seem to lead to the most maximal inhibition in the HUVEC assay and although they apparently out-performed the Avastin group, the confidence interval overlapped the zero reference so that there was no statistically significant difference from Avastin, data not shown (**Table 14A**).

Table 14B: Geometric means of IC50 for anti-VEGF dAb-Fc-dAbs with 95% confidence intervals (CI) compared to DMS30026, DMS30030 and Bevacizumab (Avastin) in the HUVEC Assay.

Predicted Means for IC50			
mAb	Estimate	Lower 95% CI	Upper 95% CI
Avastin	1.773E-9	1.504E-9	2.09E-9
DMS30022	9.03E-10	6.53E-10	1.25E-9
DMS30023	5.98E-10	4.27E-10	8.36E-10
DMS30026	1.675E-9	1.201E-9	2.336E-9
DMS30030	2.405E-9	1.82E-9	3.18E-9
DMS30034	1.216E-9	7.77E-10	1.902E-9
DMS30036	1.052E-9	8.4E-10	1.316E-9
DMS30037	8.04E-10	6.42E-10	1.007E-9

A similar analysis of the geometric means of the IC50 values with 95% confidence intervals, (CI), showed that the molecules DMS30022, DMS30023, DMS30036 and DMS30037 had statistically significantly lower IC50 values than Avastin, data not shown (**Table 14B**).

A separate set of data was generated from HUVEC assays in a similar format from a different operator. The data focussed upon comparing the behaviour of two dAb-Fc-dAbs in the HUVEC assay: DMS30022 and DMS30037 with that of Bevacizumab (Avastin) and was performed in quadruplicate. The data suggest that both dAb-Fc-dAbs have very similar IC50 values and levels of maximal inhibition in the HUVEC assay and appear more potent, though not statistically so in this particular data set, than Bevacizumab (Avastin), (data not shown).,

Example 42 – Performance of anti-VEGF Vh/Vk & Vk/Vk dAb-Fc-dAb molecules with modified linkers in VEGF induced blood-retinal breakdown: rabbit retinal leakage model

DMS30022, DMS30030, DMS30034, DMS30036 and DMS30037 were tested in a human VEGF165 (R&D Systems), induced blood-retinal breakdown, (BRB), model in the rabbit eye and compared against Bevacizumab (Avastin), and Kenacort (Tramcinalone) as described in **Example 9.**

The aim of this study and method were similar to those outlined for **Example 9** above. The dosing and injection schedule is shown in **Table 15A.**
All molecules were buffer exchanged into 50mM sodium acetate buffer pH5.5, 104mM NaCl, 0.02mM EDTA following the method of **Example 9** above.

One hundred and forty (140) HY79B pigmented rabbits were randomly divided into eleven (11) groups of twelve (12) animals and one (1) group of eight animals, each group was sub-divided into 4 experimental sets of 3 animals, bar the Kenacort treated group, (4 sets of 2 animals), (see **Table 15A**).

Mean intravitreal levels of some of the dosed molecules, (DMS30036, DMS30037 and DMS30022), were determined by an MSD, (Mesoscale Discovery), based functional VEGF binding assay, (data not shown). For the molecules measured, levels were similar to that expected from the injected levels and the likely half life range for the molecules in the rabbit vitreous, (data not shown). Fluorescein angiograms were collected for qualitative assessment.

Table 15A: Dosing and injection schedule for inhibition of VEGF induced rabbit retinal leakage by DMS30022, DMS30030, DMS30034, DMS30036 and DMS30037 compared to Bevacizumab (Avastin) and Kenacort (Triamcinalone).

Treatment (right eye)	Dose eye	Treatment Protocol	Number of animals				Induction	Measurements
			Set 1	Set 2	Set 3	Set 4		
Vehicle	-	50-µL IVT Day -7	3	3	3	3	Day 0 500 ng/50 µL rhVEGF (IVT)	*On Day 2*: * Retinal angiography assessment using Heidelberg's Retinal Angiograph * Fluorescein leakage quantification in vitreous/retina segment (Fluorotron® Master) * Eyeballs sampling (except for Kenacort group)
Avastin	H		3	3	3	3		
Avastin	L		3	3	3	3		
Avastin	L/3		3	3	3	3		
DMS30030	L		3	3	3	3		
DMS30030	L/3		3	3	3	3		
DMS30034	L/3		3	3	3	3		
DMS30036	L/3		3	3	3	3		
DMS30037	L		3	3	3	3		
DMS30037	L/3		3	3	3	3		
DMS30022	L/3		3	3	3	3		
Kenacort®Retard	2 000 ug/eye		2	2	2	2		

Intravitreally injected VEGF induced a breakdown of the BRB, which was blocked by treatment with the following compounds when un-masked: Bevacizumab (Avastin), at all three doses; DMS30030, (L and L/3 doses); DMS30022 (L/3 dose), DMS30034, (L/3 dose), DMS30036 (L dose), and DMS30037 (L and L/3 dose), 9 days post injection, with an efficacy similar to that of the marketed reference (Kenacort®). In the masked study, an important retinal vascular leakage was noted in right induced eyes after treatment with Vehicle, negative control, and DMS30036 at L/3 dose, although the classification was influenced by the presence of a single significant outling data point, (data not shown). Note than one data point (rabbit 134) was excluded from further analysis from the DMS30037 L/3 dosed group due to a abnormality scoring for fluorescence.

The raw data was subject to further statistical analysis: the masked groups corresponding to the same molecule and dose were pooled and geometric mean values were determined with 95% confidence intervals

(CI). The geometric mean data is shown Table 15B (i) and the ratio to vehicle values and variance with 95% CI are shown in Table 15B (ii). From the data analysis in Tables 15B (i) and (ii) the marketed corticoid reference (Kenacort® retard) reduced the degree of VEGF induced retinal leakage by 80%), the three different doses of Bevacizumab (Avastin), were effective by 71.4-76.4%, with no evidendce of a dose response, DMS30022 (L/3) was effective by 74.6%, DMS30037 (L) by 74.2% and (L/3) by 67.4%; DMS30036 L/3 by 63.7%; DMS30034 L/3 by 59.5% and DMS 30030 (L) by 65.5% and L/3 by 70.5%. It was concluded that Vh/Vk dAb-Fc-dAbs such as DMS30022 and DMS30037 were the most potent format in this model.

Table 15B: Inhibition of VEGF induced rabbit retinal leakage by DMS30022, DMS30030, DMS30034, DMS30036 and DMS30037 compared to Bevacizumab (Avastin) and Kenacort (Triamcinalone)

Analysis of log10 right values (RE) adjusted for log10 left eye values (LE), (excluding rabbit 134 opaque lens in group 5: DMS30037 L/3)

(i) Geometric means

grpno	group	Geometric mean	lower	upper
1	DMS30030 L/3	15070.42	10613.18	21399.57
2	Avastin L/3	14592.62	10277.06	20720.37
3	Avastin H	12332.94	8673.32	17536.69
4	DMS30036 L/3	18539.81	13041.82	26355.55
5	DMS30037 L/3	16602.99	11620.75	23721.32
6	Vehicle	51006.20	35920.69	72427.12
7	DMS30022 L/3	12975.83	9135.26	18431.04
8	DMS30037 L	13159.70	9257.22	18707.31
9	Avastin L	12037.98	8470.04	17108.87
10	DMS30030 L	17586.48	12379.27	24984.06
11	DMS30034 L/3	20632.66	14441.37	29478.27
12	Kenacort	10205.21	6929.09	15030.31

(ii) Ratio to vehicle and percentage reduction.

grpno	group	ratio	lower	upper	P value	%reduction
1	DMS30030 L/3	0.29546	0.20895	0.41779	<.0001	70.4537
2	Avastin L/3	0.28609	0.20235	0.40449	<.0001	71.3905
3	Avastin H	0.24179	0.17060	0.34269	<.0001	75.8207
4	DMS30036 L/3	0.36348	0.25683	0.51442	<.0001	63.6519
5	DMS30037 L/3	0.32551	0.22839	0.46394	<.0001	67.4491
7	DMS30022 L/3	0.25440	0.17990	0.35973	<.0001	74.5603
8	DMS30037 L	0.25800	0.18230	0.36514	<.0001	74.1998
9	Avastin L	0.23601	0.16665	0.33424	<.0001	76.3990
10	DMS30030 L	0.34479	0.24379	0.48763	<.0001	65.5209
11	DMS30034 L/3	0.40451	0.28368	0.57680	<.0001	59.5487
12	Kenacort	0.20008	0.13518	0.29612	<.0001	79.9922

Example 43 – Larger scale Purification of Vh/Vk dAb-Fc-dAb molecules from Chinese Hamster Ovary (CHO) cells

For example, DMS30037 was captured from clarified cell culture supernatant using affinity chromatography and an automated FPLC purification system. Once loaded, the bound product was washed using a combination of pH neutral aqueous buffers to remove non-specifically bound impurities followed by a low pH elution from which over 90% of bound product was recovered. After storage at -40^{0}C the elution pool was thawed then adjusted to pH 3.6 for 30 minutes to achieve virus neutralisation after which time the pH was adjusted to pH6.0. The pH adjusted pool was further purified on a second column whereby product was loaded under non-product binding conditions that promote removal of process impurities. The purified DMS30037 was then collected as a pool before 0.2µm filtration and storage. Additionally, DMS30037 affinity column eluates were pH adjusted to 7.5 in low salt buffer, filtered and then concentrated and diafiltered into a suitable formulation buffer using a tangential flow filtration system. Recovered product was 0.2µm sterile filtered and stored.

Example 44 – Cloning of anti-VEGF Vh/Vk dAb-Fc-dAb molecules with modified C-termini

The Vh-Vk dAb-Fc-dAbs with modifications to the C-terminus of the Vk dAb : DMS30047-30054 (SEQ ID NO:147-154 & 185-192, Table 19) were engineered by generating the variant Vk dAb sequences by PCR and then by re-cloning into DMS30045 and DMS30046, respectively to generate the modified mammalian expression vectors. From DMS30045: (i) the C-terminal arginine residue was removed to generate DMS30047 (DMS30037-R), (ii) a C-terminal alanine was added to generate DMS30048, (DMS30037+A), (iii) three C-terminal alanines were added to generate DMS30049, (DMS30037+AAA) and a C-terminal threonine was added to generate DMS30050 (DMS30037+T). From DMS30046: (i) the C-terminal arginine residue was removed to generate DMS30051 (DMS30038-R), (ii) a C-terminal alanine was added to generate DMS30052, (DMS30038+A), (iii) three C-terminal alanines were added to generate

DMS30053, (DMS30038+AAA) and a C-terminal threonine was added to generate DMS30054 (DMS30038+T).

Example 45 – Expression of anti-VEGF Vh/Vk dAb-Fc-dAb molecules with modified C-termini (DMS30047-30054)

Expression plasmids encoding the relevant anti-VEGF dAb-Fc-dAb molecules (listed in SEQ ID NO:147-154,and 185-192, Table 19) were transiently transfected into HEK293 6E cells and expressed at 500ml scale to produce the antibody fragment molecules using the method described in **Examples 10, 21 and 36.** Expression levels of >30mg/L supernatant were routinely achieved.

Example 46 – Purification of anti-VEGF Vh/Vk dAb-Fc-dAb molecules with modified C-termini

The dAb-Fc-dAb molecules were affinity purified from the supernatants (**Example 45**), as described for **Example 37** above..

Example 47 – Molecular Analysis by Size-Exclusion Chromatography (SEC) of anti-VEGF Vh/Vk dAb-Fc-dAb molecules with modified C-termini

The molecular integrity, homogeneity and % purity of the anti-VEGF dAb-Fc-dAb molecules which had been purified as described in **Example 46** were analysed by SDS-PAGE and analytical size-exclusion chromatography (SEC) as described in **Examples 3 & 13**. The proteins were confirmed to be >95% pure target protein by SDS-PAGE and SEC prior to further analysis in biology assays.

Example 48 – Binding of anti-VEGF Vh/Vk dAb-Fc-dAb molecules with modified C-termini to VEGF on Biacore.

The binding affinity of certain anti-VEGF dAb-Fc-dAb molecules, (with small C-terminal modifications), for $VEGF_{165}$ was determined by Surface Plasmon resonance (SPR) using a Biacore T100 in a similar manner to **Examples 34 and 40**, but with minor modifications. Protein A was immobilised on a C1 chip by primary amine coupling and this surface was then used to capture the anti-VEGF constructs. Human recombinant $VEGF_{165}$ (sourced 'in house' from transient transfection of HEK293 cells) was used as the analyte at 32nM to 0.03125nM in a 4 fold dilution series. All binding curves were double referenced with a buffer injection (i.e. 0nM) and the data was fitted to 1:1 model inherent to the T100. Regeneration was carried out using 50mM NaOH. The run was carried out at 37°C, using HBS-EP as the running buffer. The data obtained is shown in Tables 16A, 16B & 16C. From the data in Table 16A, the behaviour of DMS30037 and several variants modified at the C-terminus: DMS30037+A (DMS30048), DMS30037+AAA (DMS30049), and DMS30037+T

(DMS30050) seem comparable on Biacore and the C-terminal modifications do not appear to reduce potency over parental.

A further data set is shown in **Table 16B** where the performance of both DMS30037 and DMS30038 were compared with variants modified at the C-terminus: DMS30037-R, (labelled as +R (DMS30047), DMS30037+T (DMS30050) and DMS30038-R, (labelled as +R (DMS30051) and Bevacizumab (Avastin) in the Biacore. In this data set again the behaviour of all the molecules seems comparable on Biacore and the C-terminal modifications do not appear to reduce potency over parental. Meaningful data could not be captured other than to view the curve for Avastin. A further data set is displayed in Table 16C where the molecules DMS30037 and DMS30038 were compared with variants modified at the C-terminus: DMS30037-R, (DMS30047), DMS30037+T (DMS30050), DMS30038-R, (DMS30051) and DMS30038+T (DMS30054) and Bevacizumab (Avastin). Again the behaviour of all the dAb-Fc-dAb molecules seem comparable on Biacore and the C-terminal modifications do not appear to reduce potency over parental. In this data set, see Table 16C, the Bevacizumab (Avastin) data could not be properly measured due to the off-rate being too tight.

Table 16A: Binding of the anti-VEGF dAb-Fc-dAb molecule: DMS30037 with C-terminal modifications to $VEGF_{165}$ and comparison to DMS30037.

Ligand	Ka (M-1.s-1)	Kd (s-1)	KD (M)
DMS30037	8.18E+06	4.34E-05	5.30E-12
DMS30037+A	8.25E+06	5.21E-05	6.32E-12
DMS30037+AAA	7.74E+06	5.37E-05	6.94E-12
DMS30037+T	8.03E+06	4.21E-05	5.24E-12

Table 16B: Binding of the anti-VEGF dAb-Fc-dAb molecules: DMS30037 and DMS30038 with C-terminal modifications to $VEGF_{165}$ and comparison to parental dAb-Fc-dAb and Bevacizumab(Avastin).

Ligand	Ka (M-1.s-1)	Kd (s-1)	KD (M)
DMS30037	1.04E+07	4.39E-05	4.22E-12
DMS30037+R	1.07E+07	4.22E-05	3.94E-12
DMS30037+T	1.10E+07	4.27E-05	3.90E-12
DMS30038	1.03E+07	4.79E-05	4.64E-12
DMS30038+R	1.23E+07	5.31E-05	4.31E-12
avastin	8.39E+05	Out of range	Out of range

Table 16C: Binding of the anti-VEGF dAb-Fc-dAb molecules: DMS30037 and DMS30038 with C-terminal modifications to $VEGF_{165}$ and comparison to parental dAb-Fc-dAb and Bevacizumab(Avastin).

Ligand	ka	kd	KD
DMS30037	5.60E+06	1.46E-04	2.61E-11
DMS30037 + T	5.38E+06	1.42E-04	2.64E-11
DMS30037 – R	6.97E+06	1.55E-04	2.22E-11
DMS30038	5.69E+06	1.55E-04	2.73E-11
DMS30038 – R	5.90E+06	1.58E-04	2.68E-11
DMS30038 + T	8.28E+06	1.22E-04	1.47E-11
Avastin	1.24E+06	Out of range	Out of range

Example 49 – VEGF R2 Receptor Binding Assay of anti-VEGF Vh/Vk dAb-Fc-dAb molecules with modified C-termini

The potencies of anti-VEGF Vh/Vk dAb-Fc-dAb molecules based upon DMS30037 and DMS30038, but with C-terminal modifications, were compared both to the wild type molecule and Bevacizumab (Avastin), in the VEGF receptor 2, (R2), binding assay using the modified method, i.e. with no pre-incubation, described in **Examples 7, 17 & 26 & 40.** The data is shown in **Table 17A**, all the tested dAb-Fc-dAb molecules: DMS30037, DMS30037+T (DMS30050), DMS30037-R (DMS30047), DMS30038, DMS30038-R (DMS30051), appeared to be of comparable potency and considerably more potent than Bevacizumab (Avastin), **Table 17A.** There was little variation in the maximal percentage inhibition achived by the molecules in the assay with all molecules achieving >93-98% maximal inhibition, (data not shown).

Further data was generated comparing the dAb-Fc-dAbs: DMS30038, DMS30038+T, (DMS30050) and DMS30038-R, (DMS30051) with Bevacizumab (Avastin), in the same assay format, the data is displayed in Table 17B. From the data DMS30038 and its C-terminal variants, (Table 17B), have similar potencies judged by EC50 values in the RBA assay and appear to be considerably more potent than Bevacizumab (Avastin) by this criteria. There was little variation in the maximal percentage inhibition achieved by the molecules in the assay with all molecules achieving >94% maximal inhibition, (data not shown)

Table 17A: EC_{50} values of anti-VEGF dAb-Fc-dAbs with C-terminal modifications compared to Bevacizumab (Avastin) in VEGFR2 Receptor Binding Assay.

Curve fitting and EC_{50} calculations were performed using GraphPad Prism.

VEGFR2 RBA	EC50 (g/mL)	EC50 (pM)
Avastin	1.21E-07	806
DMS30037	2.99E-09	28
DMS30037 +T	2.98E-09	28
DMS30037 -R	2.66E-09	25
DMS30038	3.37E-09	32
DMS30038 -R	3.84E-09	37

Table 17B: EC_{50} values of anti-VEGF dAb-Fc-dAbs with C-terminal modifications compared to Bevacizumab (Avastin) in VEGFR2 Receptor Binding Assay.

Curve fitting and EC_{50} calculations were performed using GraphPad Prism.

VEGF R2 RBA	EC50 (g/mL)	EC50 (pM)
Avastin	3.4E-07	2266
DMS30038	5.28E-09	50
DMS30038 +T	4.31E-09	41
DMS30038 -R	4.53E-09	43

Example 49 - Human Umbilical Vein Endothelial Cell (HUVEC) Proliferation assay: Inhibition with anti-VEGF Vh/Vk dAb-Fc-dAb molecules containing C-terminal modifications.

The abilities of dAb-Fc-dAb molecules based upon DMS30037 and DMS30038 but with C-terminal modifications: DMS30037-R (DMS30047) & DMS30037+T (DMS30050) , DMS30038-R (DMS30051) & DMS30038+T (DMS30054) to suppress proliferation of human umbilical vein endothelial cells were compared to Bevacizumab (Avastin) using the method described in **Examples 8, 18, 27 & 41** with the following deviations (i) rather than leaving the outer wells free of cells, the whole 96 well plate was used and (ii) the data was analysed using GraphPad Prism using a Sigmodial curve fit, variable slope cf a non-linear regression (variable slope). The test compounds were independently assessed on individual plates against the comparator molecule, Bevacizumab (Avastin); the assay was carried out on at least three separate occasions, with a total data set per molecule of Bevacizumab (Avastin): 15; DMS30037: 7; DMS30038: 8; DMS30037-R (DMS30047): 3; DMS30037+T (DMS30050): 4; DMS30038-R (DMS30051): 4 & DMS30038+T (DMS30054): 4, (data not shown). The focus was upon analysing both the degree of maximum inhibition and the relative EC50 values in the assay generated by certain molecules compared to that of Bevacizumab (Avastin).

The data was analysed using GraphPad Prism using a Sigmodial curve fit, variable slope cf a non-linear regression (variable slope). Individual curve fits were fitted for each molecule and at each day. Due to some

poor fitting, it was decided to introduce constraints for the curve where a plateau was not observed at the lower concentration. One plate was excluded from the analysis due to poor curve fitting despite constraints. This constraint would be equal to the mean of the points at the lowest concentration. Data was manually selected as to whether the minimum was constrained or not, and the curve fit and parameters were automatically updated based upon this criteria selection. Estimates of the curve maxima and the standard error were analysed using a weighted mixed model analysis of variance, using 1/ (standard error)^2 , [SE]^2, as a weighting. The analysis adjusted for variability between plates and days using random effects terms. From this analysis, the predicted means were estimated and comparisons were made back to Bevacizumab (Avastin,control) (**See Table 18A**). The same analysis was then performed on the log10 scale for the IC50, and the results back transformed. From this, estimates of the geometric means were generated and comparisons were made back to Bevacizumab (Avastin) in the form of a ratio to Bevacizumab (Avastin control) i.e. a ratio of 0.5 would indicate a 50% drop from Bevacizumab, (Avastin, see**Table 18B**).

Table 18A: Predicted geometric means of maximum percentage inhibition of C-terminally modified anti-VEGF dAb-Fc-dAbs with 95% confidence intervals (CI) compared to parental and Bevacizumab (Avastin) in the HUVEC Assay.

Predicted Means for Max % Inhibition			
mAb	Estimate	Lower 95% CI	Upper 95% CI
Avastin	71.0316	61.6741	80.3891
DMS30037	85.4759	74.9164	96.0354
DMS30037+T	89.9852	78.2698	101.70
DMS30037-R	82.2693	69.9929	94.5457
DMS30038	73.5602	63.7180	83.4023
DMS30038+T	79.0343	67.1904	90.8782
DMS30038-R	77.6519	65.5487	89.7550

From this analysis, molecules DMS30037, DMS30037+T and DMS30037-R seem to lead to the most maximal inhibition in the HUVEC assay and they out-performed the Avastin group, the confidence interval did not overlap the zero reference so the data was statistically significant from that of Avastin, data not shown (see Table 18A).

Table 18B: Geometric means of IC50 for C-terminally modified anti-VEGF dAb-Fc-dAbs with 95% confidence intervals (CI) compared to parental and Bevacizumab (Avastin) in the HUVEC Assay.

Geometric Means for IC50			
mAb	Estimate	Lower 95% CI	Upper 95% CI
Avastin	3.829E-9	3.119E-9	4.7E-9
DMS30037	1.903E-9	1.473E-9	2.46E-9
DMS30037+T	2.332E-9	1.758E-9	3.092E-9
DMS30037-R	7.365E-9	2.06E-10	2.639E-7
DMS30038	2.163E-9	1.723E-9	2.715E-9
DMS30038+T	2.649E-9	1.877E-9	3.738E-9
DMS30038-R	2.234E-9	1.699E-9	2.936E-9

A similar analysis of the geometric means of the IC50 values with 95% confidence intervals, (CI), showed that almost all the dAb-Fc-dAb molecules DMS30037, DMS30037+T, DMS30038, DMS30038+T and DMS30038-R had statistically significantly lower IC50 values than Bevacizumab (Avastin), data not shown (see Table 18B). The data set from DMS30037-R was highly variable with a low n number (3).

Overall the data suggest that C-terminal modifications to both dAb-Fc-dAbs: DMS30037 & DMS30038 have very similar IC50 values and levels of maximal inhibition in the HUVEC assay to parental molecules and appear more potent, than Bevacizumab (Avastin), both interms of maximal percentage inhibition and lower IC50 , (see **Tables 18A and 18B**).

Example 50: Cross-reactivity of Vh dAb-Fc (DMS1529) and Vk Fc-dAb (DMS30000) to murine $VEGF_{164}$

A MSD, (MesoScale Discovery), high bind 96 well plate (MSD L11XB-3) was coated with 1 or 2µg/mL $mVEGF_{164}$ (R&D 493-MV/CF) (25µL/well), sealed and incubated overnight at 4°C. Next day the MSD plate was washed 3x300µL/well with Tris wash buffer and blocked with 3% BSA in PBS (250uL/well) and incubated shaking (750 RPM) at room temperature for 1 hour. The MSD plate was washed again before the addition of the DMS1529, DMS30000 or mVEGF R2 Fc (R&D 443-KD/CF) standard curves (25uL/well) and incubated shaking (750 RPM) at room temperature for 2 hours. The standards were diluted using 0.1% BSA in PBS. The MSD plate was washed again before the addition of the detection reagent (25µL/well, in-house sulfo-TAG labelled goat anti-human IgG, Fc specific – Sigma I2136) at 1µg/mL in 1% BSA in PBS and incubated shaking (750 RPM) at room temperature for 1 hour. Prior to measuring the electrochemical luminescence in a MSD Sector Imager 6000, the MSD plate was washed again and 150uL/well of 2x Read Buffer T (MSD R92TC-1) was added. Excel was used for data analysis and graph plotting.

An example data set is shown in **Figure 4** where the MSD signal is plotted against antibody concentration for DMS1529, DMS3000 and a mouse VEGF R2 Fc control protein.

There is clear binding of all 3 proteins to mouse $VEGF_{164}$ in a concentration dependent manner indicating that the dAbs cross-react with mouse $VEGF_{164}$.

Table 19 : DNA and Amino Acid Sequence ID table

Molecule ID	Molecule Description	DNA Sequence SEQ ID No.	Amino Acid Sequence SEQ ID No.
Vh dAb-Fc Sequences			
DMS1529	DOM15-26-593-Fc dAb-Fc	1 Also see Fig 52C in WO2008/149150/A2.	29 Also see Fig 52A in WO2008/149150/A2. SEQ ID1
DMS1576	DOM15-26-597-Fc dAb-Fc	2	30
Vk dAb-Fc and Fc-dAb Sequences			
DMS30000	C-terminal Fc fusion of K-044-085 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb	3	31
DMS30001	C-terminal Fc fusion of K-044-232 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb	4	32
DMS30002	C-terminal Fc fusion of K-044-236 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb	5	33
DMS30003	C-terminal Fc fusion of K-044-251 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb	6	34
DMS30004	C-terminal Fc fusion of K-044-255 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb	7	35
DMS30005	N-terminal Fc fusion of K-044-251 dAb (AAAS Linker) dAb-Fc	8	36
DMS30006	N-terminal Fc fusion of K-044-255 dAb (AAAS Linker) dAb-Fc	9	37
DMS30013	C-terminal Fc fusion of K -044-085 dAb (Albumin Domain 3 Linker) Fc-dAb	16	44
DMS30014	C-terminal Fc fusion of K -044-085 dAb (Albumin Domain 3 Linker) Fc-dAb	17	45
DMS30015	C-terminal Fc fusion of K -044-251 dAb (Albumin Domain 3 Linker) Fc-	18	46

	dAb		
DMS30016	C-terminal Fc fusion of K -044-251 dAb (Albumin Domain 3 Linker) Fc-dAb	19	47
DMS30017	N-terminal Fc fusion of K-044-085 dAb (TVAAPS Linker) dAb-Fc	20	48
DMS30018	N-terminal Fc fusion of K-044-232 dAb (TVAAPS Linker) dAb-Fc	21	49
DMS30019	N-terminal Fc fusion of K-044-236 dAb (TVAAPS Linker) dAb-Fc	22	50
DMS30020	N-terminal Fc fusion of K-044-251 dAb (TVAAPS Linker) dAb-Fc	23	51
DMS30021	N-terminal Fc fusion of K-044-255 dAb (TVAAPS Linker) dAb-Fc	24	52
Vh-Vk and Vk-Vk DAb-Fc-dAb Sequences			
DMS30007	DOM15-26-597-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-085 Vh-Vk DAb-Fc-dAb	10	38
DMS30008	DOM15-26-597-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-251 Vh-Vk DAb-Fc-dAb	11	39
DMS30009	DT02-K-044-085-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-085 Vk-Vk DAb-Fc-dAb	12	40
DMS30010	DT02-K-044-085-AAAS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-085 Vk-Vk DAb-Fc-dAb	13	41
DMS30011	DT02-K-044-251-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-251 Vk-Vk DAb-Fc-dAb	14	42
DMS30012	DT02-K-044-251-AAAS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-251 Vk-Vk DAb-Fc-dAb	15	43
DMS30022	DOM15-26-597-AS Linker-Fc-GS(TVAAPSGS)x3 Linker-DT02-K-044-	25	53

	085 Vh-Vk DAb-Fc-dAb		
DMS30023	DT02-K-044-085-HingeIgG1Linker-Fc-GS(TVAAPSGS)x3 Linker-DT02-K-044-085 Vh-Vk DAb-Fc-dAb	26	54
DMS30024	DT02-K-044-085-AS-Fc (H112A)-GS(TVAAPSGS)x3 Linker-DT02-K-044-085 Vh-Vk DAb-Fc-dAb	27	55
DMS30025	DT02-K-044-085-AS-Fc (T113P)-GS(TVAAPSGS)x3 Linker-DT02-K-044-085 Vh-Vk DAb-Fc-dAb	28	56
Linker Sequences			
AAAS Linker	N-terminal Linker	57	76
AS Linker	N-terminal Linker	58	77
TVAAPS Linker	N-terminal Linker	59	78
Hinge IgG1 Linker	N-terminal Linker	60	79
Hinge IgG3 Linker	N-terminal Linker	61	80
Fibronectin x3 Linker	N-terminal Linker	62	81
Fibronectin x4 Linker	N-terminal Linker	63	82
((GS(TVAAPSGS)x3)) Linker	C-terminal Linker	66	85
Fibronectin x3 Linker	C-terminal Linker	67	86
Fibronectin x4 Linker	C-terminal Linker	68	87
Albumin Domain 1 Linker	C-terminal Linker	69	88
Albumin Domain 2 Linker	C-terminal Linker	70	89
Albumin Domain 3 Linker	C-terminal Linker	71	90
Truncated Albumin Domain3 Linker; Alb Dom 3 -TFHAD	C-terminal Linker	72	91
Gly4Ser 3x Linker	C-terminal Linker	73	92
Gly4Ser 4x Linker	C-terminal Linker	74	93
Helical Linker	C-terminal Linker	75	94
dAb Sequences			
DOM15-26-593		95	103
DOM15-26-597		96	104
DT02-K-044-085		97	105
DT02-K-044-232		98	106

DT02-K-044-236		99	107
DT02-K-044-251		100	108
DT02-K-044-255		101	109
Fc Sequences			
Fc IgG1		102	110
H2A IgG1 Fc		64	83
T3P IgG1 Fc		65	84
CDR Sequences (According to Kabat)			
DOM15-26-593 CDR1			111
DOM15-26-593 CDR2			112
DOM15-26-593 CDR3			113
DOM15-26-597 CDR1			114
DOM15-26-597 CDR2			115
DOM15-26-597 CDR3			116
DT02-K-044-085 CDR1			117
DT02-K-044-085 CDR2			118
DT02-K-044-085 CDR3			119
DT02-K-044-232 CDR1			120
DT02-K-044-232 CDR2			121
DT02-K-044-232 CDR3			122
DT02-K-044-236 CDR1			123
DT02-K-044-236 CDR2			124
DT02-K-044-236 CDR3			125
DT02-K-044-251 CDR1			126
DT02-K-044-251 CDR2			127
DT02-K-044-251 CDR3			128
DT02-K-044-255 CDR1			129
DT02-K-044-255 CDR2			130
DT02-K-044-255 CDR3			131

Table 19 continued : DNA and Amino Acid Sequence ID table

Molecule ID	Molecule Description	DNA Sequence SEQ ID No.	Amino Acid Sequence SEQ ID No.

Vh dAb-Fc Sequences			
DMS30030	N-terminal Fc fusion of DOM15-26-597 dAb ((TGLDSP)x3)	132	155
DMS30031	N-terminal Fc fusion of DOM15-26-597 dAb ((TGLDSP)x4)		156
DMS30032	N-terminal Fc fusion of DOM15-26-597 dAb ((TGLDSP)x3 T113P mutation Fc)	133	157
DMS30033	N-terminal Fc fusion of DOM15-26-597 dAb ((TGLDSP)x4 T113P mutation Fc)	134	158
DMS30042	N-terminal Fc fusion of DOM15-26-597 dAb (IgG1 Hinge)		159
N597 IgG3	N-terminal Fc fusion of 15-26-597 dAb (IgG3 Hinge)		160
N597 TVAAPS	N-terminal Fc fusion of 15-26-597 dAb (TVAAPS)		161
Vk dAb-Fc and Fc-dAb Sequences			
DMS30026	C-terminal Fc fusion of K-044-085 dAb ((TGLDSP)x4)		162
DMS30027	C-terminal Fc fusion of K-044-085 dAb (Helical Linker)		163
DMS30028	N-terminal Fc fusion of K-044-085 dAb (IgG1 Hinge)		164
DMS30029	N-terminal Fc fusion of K-044-085 dAb (ASTHP linker)		165
N085 fib x4	N-terminal Fc fusion of K-044-085 dAb ((TGLDSP)x4)		166
N085 TVAAPS	N-terminal Fc fusion of K-044-085 dAb (TVAAPS)		167
N085 fib x3	N-terminal Fc fusion of K-044-085 dAb ((TGLDSP)x3)		168

N085 IgG3	N-terminal Fc fusion of K-044-085 dAb (IgG3 Hinge)		169
N085 H2A	N-terminal Fc fusion of K-044-085-AS-Fc (H112A)		170
C085 fib x3	C-terminal Fc fusion of K-044-085 dAb ((TGLDSP)x3)		171
C085 alb d 1	C-terminal Fc fusion of K-044-085 dAb (Albumin Domain 1)		172
C085 alb d 2	C-terminal Fc fusion of K-044-085 dAb (Albumin Domain 2)		173
C085 alb d 3-TFHAD	C-terminal Fc fusion of K-044-085 dAb (Albumin Domain 3-TFHAD)		174
C085 G4Sx3	C-terminal Fc fusion of K-044-085 dAb ((Gly4Ser)x3)		175
C085 G4Sx4	C-terminal Fc fusion of K-044-085 dAb ((Gly4Ser)x4)		176
Vh-Vk and Vk-Vk DAb-Fc-dAb Sequences			
DMS30034	K-044-085 dAb N-(VEPKSSDK linker) & C-terminal ((TGLDSP)x4)	135	177
DMS30035	K-044-085 dAb N-(ASTHP linker) & C-terminal ((TGLDSP)x4)	136	178
DMS30036	DOM15-26-597 dAb N-((TGLDSP)x3) & C-terminal K-044-085 dAb ((TGLDSP)x4)	137	179
DMS30037	DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb ((TGLDSP)x4)	138	180
DMS30038	DMS1576 with C-terminal K-044-085 dAb ((TGLDSP)x4)	139	181
DMS30039	DOM15-26-597 dAb N-((TGLDSP)x4) & C-terminal K-044-085 dAb ((TGLDSP)x4)	140	182
DMS30040	DOM15-26-597 dAb N-((TGLDSP)x3 T113P mutation Fc) & C-terminal K-	141	183

	044-085 dAb ((TGLDSP)x4)		
DMS30041	DOM15-26-597 dAb N-((TGLDSP)x4 T113P mutation Fc) & C-terminal K-044-085 dAb ((TGLDSP)x4)	142	184
DMS30043	K-044-085 dAb N-(VEPKSSDK linker) & C-terminal ((TGLDSP)x4) Codon optimised	143	177
DMS30044	DOM15-26-597 dAb N-((TGLDSP)x3) & C-terminal K-044-085 dAb ((TGLDSP)x4) Codon optimised	144	179
DMS30045	DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb ((TGLDSP)x4) Codon optimised	145	180
DMS30046	DMS1576 with C-terminal K-044-085 dAb ((TGLDSP)x4) Codon optimised	146	181
DMS30047	DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb minus C-term R ((TGLDSP)x4) Codon optimised	147	185
DMS30048	DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb + A ((TGLDSP)x4) Codon optimised	148	186
DMS30049	DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb +AAA ((TGLDSP)x4) Codon optimised	149	187
DMS30050	DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb +T ((TGLDSP)x4) Codon optimised	150	188
DMS30051	DMS1576 with C-terminal K-044-085 dAb minus C-term R ((TGLDSP)x4) Codon optimised	151	189

DMS30052	DMS1576 with C-terminal K-044-085 dAb +A ((TGLDSP)x4) Codon optimised	152	190
DMS30053	DMS1576 with C-terminal K-044-085 dAb +AAA ((TGLDSP)x4) Codon optimised	153	191
DMS30054	DMS1576 with C-terminal K-044-085 dAb +T ((TGLDSP)x4) Codon optimised	154	192

SEQUENCE LISTING

SEQ ID NO:1 DMS1529 (DOM15-26-593-Fc dAb-Fc)

GAGGTGCAGCTGTTGGTGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGTCTCTCCTGTGCAGCCTCCGGA
TTCACCTTTAAGGCTTATCCGATGATGTGGGTCCGCCAGGCTCCAGGGAAGGGTCTAGAGTGGGTTTCAGAGATTTCG
CCTTCGGGTTCTTATACATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCCGCGACAATTCCAAGAACACGC
TGTATCTGCAAATGAACAGCCTGCGTGCCGAGGACACCGCGGTATATTACTGTGCGAAAGATCCTCGGAAGTTAGACT
ACTGGGGTCAGGGAACCCTGGTCACCGTCTCGAGCGCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCT
GGGCGGACCTAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTG
CGTGGTGGTGGATGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACG
CCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGTGCTGCACCAGGATT
GGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAG
GCCAAGGGCCAGCCTAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCC
CTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAA
CTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGAGCAG
ATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGTCTGA
GCCTGTCCCCTGGCAAG

SEQ ID NO:2 DMS1576 (DOM15-26-597-Fc dAb-Fc)

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCG
GCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGAGATCA
GCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAAC
ACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACCCCCGGAAGCTG
GACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCTGAG
CTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAGGTG
ACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCTGAGGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGC
ACAACGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCAC
CAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCATC
AGCAAGGCCAAGGGCCAGCCCAGGGAACCCCAGGTGTACACCCTGCCCCCCAGCCGGGACGAGCTGACCAAGAACCA
GGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCG
AGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACA
AGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAG
AGCCTGAGCCTGTCCCCCGGCAAG

SEQ ID NO:3 DMS30000 (C-terminal Fc fusion of K-044-085 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb)

CAGGCTAGCTCTGACAAGACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGT
TCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACG
AGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGA
GCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACA
AGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAG
CCCCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGC
TTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
CTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTT
CAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGGATC
TACCGTGGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAACAGTAGCTGCTCCTTCTGGATCCGACATC
CAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGA
TTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCA
AAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAA
GATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC
GT

SEQ ID NO:4 DMS30001 (C-terminal Fc fusion of K-044-232 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb)

CAGGCTAGCTCTGACAAGACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGT
TCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACG
AGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGA
GCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACA
AGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAG
CCCCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGC
TTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
CTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTT
CAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGGATC
TACCGTGGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAACAGTAGCTGCTCCTTCTGGATCCGACATC
CAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGA
TTGGTCCTGAGTTAAGTTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCA
AAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAA

GATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC
GT

SEQ ID NO:5 DMS30002 (C-terminal Fc fusion of K-044-236 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb)

CAGGCTAGCTCTGACAAGACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGT
TCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACG
AGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGA
GCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACA
AGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAG
CCCCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGC
TTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
CTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTT
CAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGGATC
TACCGTGGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAACAGTAGCTGCTCCTTCTGGATCCGACATC
CAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGA
TTGGTCCTGAGTTAAGTTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCA
AAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAA
GATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTAAGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC
GT

SEQ ID NO:6 DMS30003 (C-terminal Fc fusion of K-044-251 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb)

CAGGCTAGCTCTGACAAGACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGT
TCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACG
AGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGA
GCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACA
AGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAG
CCCCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGC
TTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
CTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTT
CAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGGATC
TACCGTGGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAACAGTAGCTGCTCCTTCTGGATCCGACATC
CAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGA

TTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCA
AAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAA
GATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC
GT

SEQ ID NO:7 DMS30004 (C-terminal Fc fusion of K-044-255 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb)

CAGGCTAGCTCTGACAAGACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGT
TCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACG
AGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGA
GCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACA
AGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAG
CCCCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGC
TTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTG
CTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTT
CAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGGATC
TACCGTGGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAACAGTAGCTGCTCCTTCTGGATCCGACATC
CAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGA
TTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCA
AAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAA
GATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTAAGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC
GT

SEQ ID NO:8 DMS30005 (N-terminal Fc fusion of K-044-251 dAb (AAAS Linker) dAb-Fc)

GAGTCGACGGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCC
GGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATC
ATGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAG
CAGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACC
AAGGTGGAAATCAAACGGGCGGCCGCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGCGGACCT
AGCGTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTG
GATGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAA
GCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGTGCTGCACCAGGATTGGCTGAACG
GCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGC
CAGCCTAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTC
TGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACC

ACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAG
GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCT
GGCAAG

SEQ ID NO:9 DMS30006 (N-terminal Fc fusion of K-044-255 dAb (AAAS Linker) dAb-Fc)

GAGTCGACGGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCC
GGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATC
ATGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAG
CAGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTAAGACGTTCGGCCAAGGGACC
AAGGTGGAAATCAAACGGGCGGCCGCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGCGGACCT
AGCGTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTG
GATGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAA
GCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGTGCTGCACCAGGATTGGCTGAACG
GCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGC
CAGCCTAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTC
TGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACC
ACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAG
GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCT
GGCAAG

SEQ ID NO:10 DMS30007 (DOM15-26-597-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-085 Vh-Vk dAb-Fc-dAb)

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCG
GCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGAGATCA
GCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAAC
ACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACCCCCGGAAGCTG
GACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAG
CTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTG
ACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA
CAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACC
AGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCA
GCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAG
GTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGA
GAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAA
GAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGA

GCCTGAGCCTGTCCCCTGGCAAGGAGGTGGACGAGACCTACGTGCCCAAGGAGTTCAACGCCGAGACCTTCACCTTCC
ACGCCGACGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCG
GGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCA
TGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGC
AGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAA
GGTGGAAATCAAACGT

SEQ ID NO:11 DMS30008 (DOM15-26-597-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-251 Vh-Vk dAb-Fc-dAb)

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCG
GCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGAGATCA
GCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAAC
ACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACCCCCGGAAGCTG
GACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAG
CTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTG
ACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA
CAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACC
AGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCA
GCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAG
GTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGA
GAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAA
GAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGA
GCCTGAGCCTGTCCCCTGGCAAGGAGGTGGACGAGACCTACGTGCCCAAGGAGTTCAACGCCGAGACCTTCACCTTCC
ACGCCGACGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCG
GGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCA
TGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGC
AGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCA
AGGTGGAAATCAAACGT

SEQ ID NO:12 DMS30009 (DT02-K-044-085-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-085 Vk-Vk dAb-Fc-dAb)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAA

ATCAAACGTGCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCC
CCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAG
GACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGC
AGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAG
TGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCC
CAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTC
TACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCT
GGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCA
GCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGAGGTGG
ACGAGACCTACGTGCCCAAGGAGTTCAACGCCGAGACCTTCACCTTCCACGCCGACGACATCCAGATGACCCAGTCTCC
ATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAG
TGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCAC
GTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTACTA
CTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:13 DMS30010 (DT02-K-044-085-AAAS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-085 Vk-Vk dAb-Fc-dAb)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAA
ATCAAACGTGCCGCTGCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCC
TGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCC
ACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGA
GGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGT
ACAAGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGA
GAGCCCCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAG
GGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCT
GTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGT
GTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGA
GGTGGACGAGACCTACGTGCCCAAGGAGTTCAACGCCGAGACCTTCACCTTCCACGCCGACGACATCCAGATGACCCA
GTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAG
TTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCC
CATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTAC
GTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:14 DMS30011 (DT02-K-044-251-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-251 Vk-Vk dAb-Fc-dAb)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAA
ATCAAACGTGCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCC
CCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAG
GACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGC
AGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAG
TGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCC
CAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTC
TACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCT
GGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCA
GCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGAGGTGG
ACGAGACCTACGTGCCCAAGGAGTTCAACGCCGAGACCTTCACCTTCCACGCCGACGACATCCAGATGACCCAGTCTCC
ATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAG
TGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCAC
GTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTACTA
CTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:15 DMS30012 (DT02-K-044-251-AAAS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-251 Vk-Vk d-Fc-dAb)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAA
ATCAAACGTGCCGCTGCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCC
TGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCC
ACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGA
GGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGT
ACAAGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGA
GAGCCCCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAG
GGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCT
GTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGT

GTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGA
GGTGGACGAGACCTACGTGCCCAAGGAGTTCAACGCCGAGACCTTCACCTTCCACGCCGACGACATCCAGATGACCCA
GTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAG
TTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCC
CATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTAC
GTACTACTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:16 DMS30013 (C-terminal Fc fusion of K-044-085 dAb (Albumin Domain 3 Linker) Fc-dAb)

GCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGC
CTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAG
GTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACA
GCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTG
TCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTAC
ACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGC
GACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGA
TGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGT
GATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGAGGTGGACGAGACCT
ACGTGCCCAAGGAGTTCAACGCCGAGACCTTCACCTTCCACGCCGACGACATCCAGATGACCCAGTCTCCATCCTCCCT
GTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCA
GCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGT
GGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAAC
AGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:17 DMS30014 (C-terminal Fc fusion of K-044-085 dAb (Albumin Domain 3 Linker) Fc-dAb)

ACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGG
ACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAGGTGAAG
TTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTA
CCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACA
AGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTGC
CCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGC
TTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGTGATGCAC
GAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGAGGTGGACGAGACCTACGTGCCC

AAGGAGTTCAACGCCGAGACCTTCACCTTCCACGCCGACGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATC
TGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACC
AGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGA
TCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTA
TTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:18 DMS30015 (C-terminal Fc fusion of K-044-251 dAb (Albumin Domain 3 Linker) Fc-dAb)

GCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGC
CTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAG
GTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACA
GCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTG
TCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTAC
ACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGC
GACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGA
TGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGT
GATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGAGGTGGACGAGACCT
ACGTGCCCAAGGAGTTCAACGCCGAGACCTTCACCTTCCACGCCGACGACATCCAGATGACCCAGTCTCCATCCTCCCT
GTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCA
GCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGT
GGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAAC
AGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:19 DMS30016 (C-terminal Fc fusion of K-044-251 dAb (Albumin Domain 3 Linker) Fc-dAb)

ACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGG
ACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAGGTGAAG
TTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTA
CCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACA
AGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTGC
CCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGC
TTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGTGATGCAC
GAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGAGGTGGACGAGACCTACGTGCCC
AAGGAGTTCAACGCCGAGACCTTCACCTTCCACGCCGACGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATC

TGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACC
AGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGA
TCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTA
TTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:20 DMS30017 (N-terminal Fc fusion of K-044-085 dAb (TVAAPS Linker) dAb-Fc)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAA
ATCAAACGTACCGTCGCCGCTCCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGCGGACCTAGCG
TGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTGGATG
TGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCC
AGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAA
GGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGC
CTAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTGGT
GAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCC
CCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCA
ACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCA
AG

SEQ ID NO:21 DMS30018 (N-terminal Fc fusion of K-044-232 dAb (TVAAPS Linker) dAb-Fc)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAGTTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCAT
TTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAA
CCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAAA
TCAAACGTACCGTCGCCGCTCCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGCGGACCTAGCGT
GTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTGGATGT
GAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCCA
GAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAG
GAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCC
TAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTG
AAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCC

CCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAA
CGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCAA
G

SEQ ID NO:22 DMS30019 (N-terminal Fc fusion of K-044-236 dAb (TVAAPS Linker) dAb-Fc)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAGTTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCAT
TTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAA
CCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTAAGACGTTCGGCCAAGGGACCAAGGTGGAAA
TCAAACGTACCGTCGCCGCTCCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGCGGACCTAGCGT
GTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTGGATGT
GAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCCA
GAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAG
GAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCC
TAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTG
AAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCC
CCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAA
CGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCAA
G

SEQ ID NO:23 DMS30020 (N-terminal Fc fusion of K-044-251 dAb (TVAAPS Linker) dAb-Fc)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAA
ATCAAACGTACCGTCGCCGCTCCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGCGGACCTAGCG
TGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTGGATG
TGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCC
AGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAA
GGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGC
CTAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTGGT
GAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCC
CCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCA

ACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCA
AG

SEQ ID NO:24 DMS30021 (N-terminal Fc fusion of K-044-255 dAb (TVAAPS Linker) dAb-Fc)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTAAGACGTTCGGCCAAGGGACCAAGGTGGAA
ATCAAACGTACCGTCGCCGCTCCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGCGGACCTAGCG
TGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTGGATG
TGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCC
AGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAA
GGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGC
CTAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTGGT
GAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCC
CCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCA
ACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCA
AG

SEQ ID NO:25 DMS30022 (DOM15-26-597-AS Linker-Fc-GS(TVAAPSGS)x3 Linker-DT02-K-044-085 Vh-Vk dAb-Fc-dAb)

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCG
GCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGAGATCA
GCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAAC
ACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACCCCCGGAAGCTG
GACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCTAGCACCCACACCTGCCCCCCCTGCCCTGCCCCCGAG
CTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTG
ACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA
CAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACC
AGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCA
GCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAG
GTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGA
GAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAA
GAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGA

GCCTGAGCCTGTCCCCTGGCAAGGGATCTACCGTGGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAA
CAGTAGCTGCTCCTTCTGGATCCGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTC
ACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAG
CTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCA
CTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCG
GCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:26 DMS30023 (DT02-K-044-085-HingeIgG1Linker-Fc-GS(TVAAPSGS)x3 Linker-DT02-K-044-085 Vh-Vk dAb-Fc-dAb)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAA
ATCAAACGTgtggagcctaagtcttctgacaagACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGC
GTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGAT
GTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCC
CAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCA
AGGAGTACAAGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAG
CCCAGAGAGCCCCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTG
GTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCAC
CCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGG
GCAACGTGTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTG
GCAAGGGATCTACCGTGGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAACAGTAGCTGCTCCTTCTG
GATCCGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGC
AAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGG
TTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGT
CTGCAACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGT
GGAAATCAAACGT

SEQ ID NO:27 DMS30024 (DT02-K-044-085-AS-Fc (H112A)-GS(TVAAPSGS)x3 Linker-DT02-K-044-085 Vh-Vk dAb-Fc-dAb)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAA

ATCAAACGTGCTAGCACCGCCACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCC
CCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAG
GACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGC
AGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAG
TGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCC
CAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTC
TACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCT
GGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCA
GCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGGATCTA
CCGTGGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAACAGTAGCTGCTCCTTCTGGATCCGACATCCA
GATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATT
GGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAA
GTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGA
TTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:28 DMS30025 (DT02-K-044-085-AS-Fc (T113P)-GS(TVAAPSGS)x3 Linker-DT02-K-044-085 Vh-Vk dAb-Fc-dAb)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAA
ATCAAACGTGCTAGCACCCACCCTTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCC
CCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAG
GACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGC
AGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAG
TGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCC
CAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTC
TACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCT
GGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCA
GCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGGATCTA
CCGTGGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAACAGTAGCTGCTCCTTCTGGATCCGACATCCA
GATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATT
GGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAA
GTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGA
TTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:29 DMS1529 (DOM15-26-593-Fc dAb-Fc)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYL
QMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK

SEQ ID NO:30 DMS1576 (DOM15-26-597-Fc dAb-Fc)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNTLYL
QMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK

SEQ ID NO:31 DMS30000 (C-terminal Fc fusion of K-044-085 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb)

QASSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAP
SGSTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:32 DMS30001 (C-terminal Fc fusion of K-044-232 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb)

QASSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAP
SGSTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELSWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYMYYPETFGQGTKVEIKR

SEQ ID NO:33 DMS30002 (C-terminal Fc fusion of K-044-236 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb)

QASSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAP
SGSTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELSWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYMYYPKTFGQGTKVEIKR

SEQ ID NO:34 DMS30003 (C-terminal Fc fusion of K-044-251 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb)

QASSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAP
SGSTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYMYYPETFGQGTKVEIKR

SEQ ID NO:35 DMS30004 (C-terminal Fc fusion of K-044-255 dAb (GS(TVAAPSGS)x3 Linker) Fc-dAb)

QASSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAP
SGSTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYMYYPKTFGQGTKVEIKR

SEQ ID NO:36 DMS30005 (N-terminal Fc fusion of K-044-251 dAb (AAAS Linker) dAb-Fc)

ESTDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQYMYYPETFGQGTKVEIKRAAASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK

SEQ ID NO:37 DMS30006 (N-terminal Fc fusion of K-044-255 dAb (AAAS Linker) dAb-Fc)

ESTDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQYMYYPKTFGQGTKVEIKRAAASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK

FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK

SEQ ID NO:38 DMS30007 (DOM15-26-597-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-085 Vh-Vk dAb-Fc-dAb)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNTLYL
QMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKEVDETYVPKEFNAETFTFHADDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAP
KLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:39 DMS30008 (DOM15-26-597-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-251 Vh-Vk dAb-Fc-dAb)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNTLYL
QMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKEVDETYVPKEFNAETFTFHADDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAP
KLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPETFGQGTKVEIKR

SEQ ID NO:40 DMS30009 (DT02-K-044-085-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-085 Vk-Vk dAb-Fc-dAb)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPHTFGQGTKVEIKRASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKEVDETYVPKEFNAETFTFHADDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:41 DMS30010 (DT02-K-044-085-AAAS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-085 Vk-Vk dAb-Fc-dAb)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPHTFGQGTKVEIKRAAASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
5 KSLSLSPGKEVDETYVPKEFNAETFTFHADDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSIL
QSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:42 DMS30011 (DT02-K-044-251-AS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-251 Vk-Vk dAb-Fc-dAb)

10

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPETFGQGTKVEIKRASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
15 SLSPGKEVDETYVPKEFNAETFTFHADDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPETFGQGTKVEIKR

SEQ ID NO:43 DMS30012 (DT02-K-044-251-AAAS Linker-Fc-Albumin Domain 3 Linker-DT02-K-044-251 Vk-Vk dAb-Fc-dAb)

20

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPETFGQGTKVEIKRAAASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
25 KSLSLSPGKEVDETYVPKEFNAETFTFHADDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSIL
QSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPETFGQGTKVEIKR

SEQ ID NO:44 DMS30013 (C-terminal Fc fusion of K-044-085 dAb (Albumin Domain 3 Linker) Fc-dAb)

30

ASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKEVDETYVPKEFNAETFTFHA
DDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDF
35 ATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:45 DMS30014 (C-terminal Fc fusion of K-044-085 dAb (Albumin Domain 3 Linker) Fc-dAb)

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKEVDETYVPKEFNAETFTFHADD
IQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFAT
YYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:46 DMS30015 (C-terminal Fc fusion of K-044-251 dAb (Albumin Domain 3 Linker) Fc-dAb)

ASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKEVDETYVPKEFNAETFTFHA
DDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDF
ATYYCQQYMYYPETFGQGTKVEIKR

SEQ ID NO:47 DMS30016 (C-terminal Fc fusion of K-044-251 dAb (Albumin Domain 3 Linker) Fc-dAb)

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKEVDETYVPKEFNAETFTFHADD
IQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFAT
YYCQQYMYYPETFGQGTKVEIKR

SEQ ID NO:48 DMS30017 (N-terminal Fc fusion of K-044-085 dAb (TVAAPS Linker) dAb-Fc)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPHTFGQGTKVEIKRTVAAPSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

SEQ ID NO:49 DMS30018 (N-terminal Fc fusion of K-044-232 dAb (TVAAPS Linker) dAb-Fc)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELSWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPETFGQGTKVEIKRTVAAPSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

SEQ ID NO:50 DMS30019 (N-terminal Fc fusion of K-044-236 dAb (TVAAPS Linker) dAb-Fc)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELSWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPKTFGQGTKVEIKRTVAAPSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

SEQ ID NO:51 DMS30020 (N-terminal Fc fusion of K-044-251 dAb (TVAAPS Linker) dAb-Fc)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPETFGQGTKVEIKRTVAAPSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

SEQ ID NO:52 DMS30021 (N-terminal Fc fusion of K-044-255 dAb (TVAAPS Linker) dAb-Fc)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPKTFGQGTKVEIKRTVAAPSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

SEQ ID NO:53 DMS30022 (DOM15-26-597-AS Linker-Fc-GS(TVAAPSGS)x3 Linker-DT02-K-044-085 Vh-Vk dAb-Fc-dAb)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNTLYL
QMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAPSGSTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQK
PGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:54 DMS30023 (DT02-K-044-085-HingeIgG1Linker-Fc-GS(TVAAPSGS)x3 Linker-DT02-K-044-085 Vh-Vk dAb-Fc-dAb)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPHTFGQGTKVEIKRVEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGKGSTVAAPSGSTVAAPSGSTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPK
LLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:55 DMS30024 (DT02-K-044-085-AS-Fc (H112A)-GS(TVAAPSGS)x3 Linker-DT02-K-044-085 Vh-Vk dAb-Fc-dAb)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPHTFGQGTKVEIKRASTATCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKGSTVAAPSGSTVAAPSGSTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYH
GSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:56 DMS30025 (DT02-K-044-085-AS-Fc (T113P)-GS(TVAAPSGS)x3 Linker-DT02-K-044-085 Vh-Vk dAb-Fc-dAb)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPHTFGQGTKVEIKRASTHPCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL

SLSPGKGSTVAAPSGSTVAAPSGSTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYH
GSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:57 AAAS Linker (N-terminal Linker)

GCGGCCGCTAGC

SEQ ID NO:58 AS Linker (N-terminal Linker)

GCTAGC

SEQ ID NO:59 TVAAPS Linker (N-terminal Linker)

ACCGTCGCCGCTCCTAGC

SEQ ID NO:60 Hinge IgG1 Linker (N-terminal Linker)

GTGGAGCCTAAGTCTTCTGACAAG

SEQ ID NO:61 Hinge IgG3 Linker (N-terminal Linker)

GAGCTCAAAACCCCACTTGGTGACACA

SEQ ID NO:62 Fibronectin x3 Linker (N-terminal Linker)

ACCGGATTAGACAGTCCCACAGGTCTCGATTCACCTACTGGCTTAGACTCTCCA

SEQ ID NO:63 Fibronectin x4 Linker (N-terminal Linker)

ACCGGATTAGACAGTCCCACAGGTCTCGATTCACCTACTGGCTTAGACTCTCCAACCGGCCTGGACAGCCCC

SEQ ID NO:64 H2A IgG1 Fc

ACCGCCACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGG
ACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAGGTGAAG
TTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTA
CCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACA

AGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTGC
CCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGC
TTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGTGATGCAC
GAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAG

SEQ ID NO:65 T3P IgG1 Fc

ACCCACCCTTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGG
ACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAGGTGAAG
TTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTA
CCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACA
AGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTGC
CCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGC
TTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGTGATGCAC
GAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAG

SEQ ID NO:66 ((GS(TVAAPSGS)x3)) Linker (C-terminal Linker)

GGATCTACCGTGGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAACAGTAGCTGCTCCTTCTGGATCC

SEQ ID NO:67 Fibronectin x3 Linker (C-terminal Linker)

ACCGGATTAGACAGTCCCACAGGTCTCGATTCACCTACTGGCTTAGACTCTCCA

SEQ ID NO:68 Fibronectin x4 Linker (C-terminal Linker)

ACCGGATTAGACAGTCCCACAGGTCTCGATTCACCTACTGGCTTAGACTCTCCAACCGGCCTGGACAGCCCC

SEQ ID NO:69 Albumin Domain 1 Linker (C-terminal Linker)

CACAAGGACGACAACCCCAACCTGCCCAGGCTGGTGAGGCCCGAGGTGGACGTGATG

SEQ ID NO:70 Albumin Domain 2 Linker (C-terminal Linker)

GAGAACGACGAGATGCCCGCCGACCTGCCCAGCCTGGCCGCCGACTTCGTGGAGAGCAAGGAC

SEQ ID NO:71 Albumin Domain 3 Linker (C-terminal Linker)

GAGGTGGACGAGACCTACGTGCCCAAGGAGTTCAACGCCGAGACCTTCACCTTCCACGCCGAC

SEQ ID NO:72 Truncated Albumin Domain3 Linker; Alb Dom 3 -TFHAD (C-terminal Linker)

GAGGTGGACGAGACCTACGTGCCCAAGGAGTTCAACGCCGAGACCTTC

SEQ ID NO:73 Gly4Ser 3x Linker (C-terminal Linker)

GGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGGTCG

SEQ ID NO:74 Gly4Ser 4x Linker (C-terminal Linker)

GGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGGTCGGGTGGAGGCGGTTCA

SEQ ID NO:75 Helical Linker (C-terminal Linker)

AAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCCGCCAAGGAGCTGGCCGCCAAGGAGGCCGCCGCC
AAGGAGGCCGCCGCCAAGGAGGCCGCCGCCAAAGAATTGGCCGCA

SEQ ID NO:76 AAAS Linker (N-terminal Linker)

AAAS

SEQ ID NO:77 AS Linker (N-terminal Linker)

AS

SEQ ID NO:78 TVAAPS Linker (N-terminal Linker)

TVAAPS

SEQ ID NO:79 Hinge IgG1 Linker (N-terminal Linker)

VEPKSSDK

SEQ ID NO:80 Hinge IgG3 Linker (N-terminal Linker)

ELKTPLGDT

SEQ ID NO:81 Fibronectin x3 Linker (N-terminal Linker)

TGLDSPTGLDSPTGLDSP

SEQ ID NO:82 Fibronectin x4 Linker (N-terminal Linker)

TGLDSPTGLDSPTGLDSPTGLDSP

SEQ ID NO:83 H2A IgG1 Fc

TATCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:84 T3P IgG1 Fc

THPCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:85 ((GS(TVAAPSGS)x3)) Linker (C-terminal Linker)

GSTVAAPSGSTVAAPSGSTVAAPSGS

SEQ ID NO:86 Fibronectin x3 Linker (C-terminal Linker)

TGLDSPTGLDSPTGLDSP

SEQ ID NO:87 Fibronectin x4 Linker (C-terminal Linker)

TGLDSPTGLDSPTGLDSPTGLDSP

SEQ ID NO:88 Albumin Domain 1 Linker (C-terminal Linker)

HKDDNPNLPRLVRPEVDVM

SEQ ID NO:89 Albumin Domain 2 Linker (C-terminal Linker)

ENDEMPADLPSLAADFVESKD

SEQ ID NO:90 Albumin Domain 3 Linker (C-terminal Linker)

EVDETYVPKEFNAETFTFHAD

SEQ ID NO:91 Truncated Albumin Domain 3 Linker; Alb Dom 3 –TFHAD (C-terminal Linker)

EVDETYVPKEFNAETF

SEQ ID NO:92 Gly4Ser 3x Linker (C-terminal Linker)

GGGGSGGGGSGGGGS

SEQ ID NO:93 Gly4Ser 4x Linker (C-terminal Linker)

GGGGSGGGGSGGGGSGGGGS

SEQ ID NO:94 Helical Linker (C-terminal Linker)

KEAAAKEAAAKEAAAKELAAKEAAAKEAAAKEAAAKELAA

SEQ ID NO:95 DOM15-26-593 dAb

GAGGTGCAGCTGTTGGTGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGTCTCTCCTGTGCAGCCTCCGGA
TTCACCTTTAAGGCTTATCCGATGATGTGGGTCCGCCAGGCTCCAGGGAAGGGTCTAGAGTGGGTTTCAGAGATTTCG
CCTTCGGGTTCTTATACATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCCGCGACAATTCCAAGAACACGC
TGTATCTGCAAATGAACAGCCTGCGTGCCGAGGACACCGCGGTATATTACTGTGCGAAAGATCCTCGGAAGTTAGACT
ACTGGGGTCAGGGAACCCTGGTCACCGTCTCGAGC

SEQ ID NO:96 DOM15-26-597 dAb

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCG
GCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGAGATCA
GCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAAC
ACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACCCCCGGAAGCTG
GACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC

SEQ ID NO:97 DT02-K-044-085 dAb

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAA
ATCAAACGT

SEQ ID NO:98 DT02-K-044-232 dAb

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAGTTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCAT
TTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAA
CCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAAA
TCAAACGT

SEQ ID NO:99 DT02-K-044-236 dAb

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAGTTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCAT
TTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAA
CCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTAAGACGTTCGGCCAAGGGACCAAGGTGGAAA
TCAAACGT

SEQ ID NO:100 DT02-K-044-251 dAb

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA

ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTGAGACGTTCGGCCAAGGGACCAAGGTGGAA
ATCAAACGT

SEQ ID NO:101 DT02-K-044-255 dAb

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTC
AGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCA
TTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCA
ACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTAAGACGTTCGGCCAAGGGACCAAGGTGGAA
ATCAAACGT

SEQ ID NO:102 Fc IgG1

ACCCACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGG
ACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAGGTGAAG
TTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTA
CCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACA
AGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTGC
CCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGC
TTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGTGATGCAC
GAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAG

SEQ ID NO:103 DOM15-26-593 dAb

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYL
QMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:104 DOM15-26-597 dAb

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNTLYL
QMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:105 DT02-K-044-085 dAb

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:106 DT02-K-044-232 dAb

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELSWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPETFGQGTKVEIKR

SEQ ID NO:107 DT02-K-044-236 dAb

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELSWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPKTFGQGTKVEIKR

SEQ ID NO:108 DT02-K-044-251 dAb

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPETFGQGTKVEIKR

SEQ ID NO:109 DT02-K-044-255 dAb

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPKTFGQGTKVEIKR

SEQ ID NO:110 Fc IgG1 dAb

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:111 DOM15-26-593 CDR1

AYPMM

SEQ ID NO:112 DOM15-26-593 CDR2

EISPSGSYTYYADSVKG

SEQ ID NO:113 DOM15-26-593 CDR3

DPRKLDY

SEQ ID NO:114 DOM15-26-597 CDR1

AYPMM

SEQ ID NO:115 DOM15-26-597 CDR2

EISPSGSNTYYADSVKG

SEQ ID NO:116 DOM15-26-597 CDR3

DPRKLDY

SEQ ID NO:117 DT02-K-044-085 CDR1

RASQWIGPELK

SEQ ID NO:118 DT02-K-044-085 CDR2

HGSILQS

SEQ ID NO:119 DT02-K-044-085 CDR3

QQYMYYPHT

SEQ ID NO:120 DT02-K-044-232 CDR1

RASQWIGPELS

SEQ ID NO:121 DT02-K-044-232 CDR2

HGSILQS

SEQ ID NO:122 DT02-K-044-232 CDR3

QQYMYYPET

SEQ ID NO:123 DT02-K-044-236 CDR1

RASQWIGPELS

SEQ ID NO:124 DT02-K-044-236 CDR2

HGSILQS

SEQ ID NO:125 DT02-K-044-236 CDR3

QQYMYYPKT

SEQ ID NO:126 DT02-K-044-251 CDR1

RASQWIGPELK

SEQ ID NO:127 DT02-K-044-251 CDR2

HGSILQS

SEQ ID NO:128 DT02-K-044-251 CDR3

QQYMYYPET

SEQ ID NO:129 DT02-K-044-255 CDR1

RASQWIGPELK

SEQ ID NO:130 DT02-K-044-255 CDR2

HGSILQS

SEQ ID NO:131 DT02-K-044-255 CDR3

QQYMYYPKT

SEQ ID NO:132 N-terminal Fc fusion of DOM15-26-597 dAb ((TGLDSP)x3).

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCTATGATGTGGGTGCGGCAGGCCCCTGGTAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCAAGCGGCAGCAACACCTACTACGCAGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACACTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCAGTGTACTACTGCGCCAAGGAC
CCACGGAAGCTGGACTACTGGGGTCAGGGCACCCTGGTGACCGTGAGCAGCACCGGATTAGACAGTCCCACAGG
TCTCGATTCACCTACTGGCTTAGACTCTCCAACCCACACCTGCCCCCCCTGCCCTGCCCCAGAGCTGCTGGGCGGAC
CTAGCGTGTTCCTGTTCCCACCAAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGT
GGTGGATGTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCA
AGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAT
TGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGC
AAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTGCCCCCTAGCAGAAATGAGCTGACCAAGAACCA
GGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGC
CCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGT
GGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACA
CCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAG

SEQ ID NO:133 N-terminal Fc fusion of DOM15-26-597 dAb (TGLDSP)x3 T113P mutation Fc).

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCACGGGTCTGGACAGTCCGACTGGT
TTAGATTCACCTACGGGCTTGGACTCCCCAACCCACCCTTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCC
CCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGT
GGTGGATGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACGCCA
AGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGTGCTGCACCAGGATT
GGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGC
AAGGCCAAGGGCCAGCCTAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAG
GTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCC
GAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACCGTGG
ACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAATCACTACACC
CAGAAGAGCCTGAGCCTGTCCCCCGGCAAG

SEQ ID NO:134 N-terminal Fc fusion of DOM15-26-597 dAb (TGLDSP)x4 T113P mutation Fc).

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCACGGGTCTGGACAGTCCGACTGGT
TTAGATTCACCTACGGGCTTGGACTCCCCAACCGGCTTAGATAGCCCGACCCACCCTTGCCCCCCCTGCCCTGCCCC
CGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCC
CGAAGTGACCTGCGTGGTGGTGGATGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCG
TGGAAGTGCACAACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTG
ACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCC
TATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGA
TGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTG
GGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCT
GTACTCCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGG
CCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCCGGCAAG

SEQ ID NO:135: K-044-085 dAb N-(VEPKSSDK linker) & C-terminal ((TGLDSP)x4)

GACATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCTCTGTGGGAGACAGGGTGACTATCACCTGCAGGGC
CAGCCAGTGGATTGGCCCCGAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACCA
CGGCAGCATCCTGCAGTCCGGCGTGCCTAGCAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTCACCAT
CAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTATATGTACTACCCCCACACCTTCGGCCAG
GGCACCAAGGTGGAGATCAAGAGGGTGGAGCCTAAGTCTTCTGACAAGACCCACACCTGCCCCCCCTGCCCTGCC
CCAGAGCTGCTGGGAGGACCCAGCGTGTTCCTGTTCCCACCCAAGCCTAAGGACACCCTGATGATCAGCAGAACC
CCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGG
CGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGC
TGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCC
CTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTGCCCCCTAGCAGA
GATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAG
TGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTC
CTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGTGATGCACGA
GGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGACCGGATTAGACAGTCCCACAGG

TCTCGATTCACCTACTGGCTTAGACTCTCCAACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGTCTCCATCCT
CCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTG
GTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCA
CGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGT
ACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:136: K-044-085 dAb N-(ASTHP linker) & C-terminal ((TGLDSP)x4)

GACATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCTCTGTGGGAGACAGGGTGACTATCACCTGCAGGGC
CAGCCAGTGGATTGGCCCCGAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACCA
CGGCAGCATCCTGCAGTCCGGCGTGCCTAGCAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTCACCAT
CAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTATATGTACTACCCCCACACCTTCGGCCAG
GGCACCAAGGTGGAGATCAAGAGGGCTAGCACCCACCCTTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGC
CCCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGG
TGGTGGATGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
AAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGTGCTGCACCAGGA
TTGGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCA
GCAAGGCCAAGGGCCAGCCTAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACC
AGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAG
CCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACCG
TGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAATCACTAC
ACCCAGAAGAGTCTGAGCCTGTCCCCTGGCAAGACCGGATTAGACAGTCCCACAGGTCTCGATTCACCTACTGGCT
TAGACTCTCCAACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA
GACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGG
AAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAT
CTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATG
TATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:137: DOM15-26-597 dAb N-((TGLDSP)x3) & C-terminal K-044-085 dAb ((TGLDSP)x4)

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCGG
CTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGAGATCAG
CCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACAC
CCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACCCCCGGAAGCTGGA
CTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCACGGGTCTGGACAGTCCGACTGGTTTAGATTCACCTACGGG

CTTGGACTCCCCAACCCACACCTGCCCCCCCTGCCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCC
CAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCC
TGAGGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAACAGTACA
ACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGG
TGTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGGGAACCCCAGGTGT
ACACCCTGCCCCCCAGCCGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAG
CGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGA
CGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGT
GATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCCCGGCAAGACCGGATTAGACAGTCCC
ACAGGTCTCGATTCACCTACTGGCTTAGACTCTCCAACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGTCTCCATC
CTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGG
TACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTT
CAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTC
AACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:138: DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb ((TGLDSP)x4)

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCTATGATGTGGGTGCGGCAGGCCCCTGGTAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCAAGCGGCAGCAACACCTACTACGCAGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACACTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCAGTGTACTACTGCGCCAAGGAC
CCACGGAAGCTGGACTACTGGGGTCAGGGCACCCTGGTGACCGTGAGCAGCGTGGAGCCTAAGTCTTCTGACAA
GACCCACACCTGCCCACCCTGCCCTGCCCCAGAGCTGCTGGGAGGACCCAGCGTGTTCCTGTTCCCACCCAAGCCT
AAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGA
GGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTAC
AACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTG
TAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCC
CCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGG
CTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACCCCCCC
TGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCA
ACGTGTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGG
CAAGACCGGATTAGACAGTCCCACAGGTCTCGATTCACCTACTGGCTTAGACTCTCCAACCGGCCTGGACAGCCCC
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAA
GTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATG
GTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAG

CAGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGA
CCAAGGTGGAAATCAAACGT

SEQ ID NO:139: DMS1576 with C-terminal K-044-085 dAb ((TGLDSP)x4)

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCTAGCACCCACACCTGCCCCCCCT
GCCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAG
CCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCTGAGGTGAAGTTCAATTGGTACG
TGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGT
GTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGCCCT
GCCTGCCCCCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGGGAACCCCAGGTGTACACCCTGCCCCC
CAGCCGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGC
CGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCA
GCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGA
TGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGACCGGATTAGACAGTC
CCACAGGTCTCGATTCACCTACTGGCTTAGACTCTCCAACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGTC
TCCATCCTCCCTGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAG
TTAAAGTGGTACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGG
GTCCCATCACGTTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATT
TTGCTACGTACTACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACG
T

SEQ ID NO:140: DOM15-26-597 dAb N-((TGLDSP)x4) & C-terminal K-044-085 dAb ((TGLDSP)x4)

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCACGGGTCTGGACAGTCCGACTGGT
TTAGATTCACCTACGGGCTTGGACTCCCCAACCGGCTTAGATAGCCCGACCCACACCTGCCCCCCCTGCCCTGCCCC
TGAGCTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCC

GAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCTGAGGTGAAGTTCAATTGGTACGTGGACGGCGT
GGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGA
CCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCCA
TCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGGGAACCCCAGGTGTACACCCTGCCCCCCAGCCGGGAC
GAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGG
GAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTG
TACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGC
CCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGACCGGATTAGACAGTCCCACAGGTCTC
GATTCACCTACTGGCTTAGACTCTCCAACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGTCTCCATCCTCCCT
GTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTA
CCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCACGT
TTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTACT
ACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:141: DOM15-26-597 dAb N-((TGLDSP)x3 T113P mutation Fc) & C-terminal K-044-085 dAb ((TGLDSP)x4)

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCACGGGTCTGGACAGTCCGACTGGT
TTAGATTCACCTACGGGCTTGGACTCCCCAACCCACCCTTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCC
CCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGT
GGTGGATGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACGCCA
AGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGTGCTGCACCAGGATT
GGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGC
AAGGCCAAGGGCCAGCCTAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAG
GTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCC
GAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACCGTGG
ACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAATCACTACACC
CAGAAGAGTCTGAGCCTGTCCCCTGGCAAGACCGGATTAGACAGTCCCACAGGTCTCGATTCACCTACTGGCTTAG
ACTCTCCAACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGA
CCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGTACCAGCAGAAACCAGGGAA
AGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCAGTGGATCT

GGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTACTACTGTCAACAGTATATGTA
TTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:142: DOM15-26-597 dAb N-((TGLDSP)x4 T113P mutation Fc) & C-terminal K-044-085 dAb ((TGLDSP)x4)

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCACGGGTCTGGACAGTCCGACTGGT
TTAGATTCACCTACGGGCTTGGACTCCCCAACCGGCTTAGATAGCCCGACCCACCCTTGCCCCCCCTGCCCTGCCCC
CGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCC
CGAAGTGACCTGCGTGGTGGTGGATGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCG
TGGAAGTGCACAACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTG
ACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGCCTGCCCC
TATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGAGAGCCCCAGGTCTACACCCTGCCTCCCTCCAGAGA
TGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTG
GGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCT
GTACTCCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGG
CCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCAAGACCGGATTAGACAGTCCCACAGGTCT
CGATTCACCTACTGGCTTAGACTCTCCAACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGTCTCCATCCTCCC
TGTCTGCATCTGTAGGAGACCGTGTCACCATCACTTGCCGGGCAAGTCAGTGGATTGGTCCTGAGTTAAAGTGGT
ACCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATCATGGTTCCATTTTGCAAAGTGGGGTCCCATCACG
TTTCAGTGGCAGTGGATCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCTACGTAC
TACTGTCAACAGTATATGTATTATCCTCATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT

SEQ ID NO:143: K-044-085 dAb N-(VEPKSSDK linker) & C-terminal ((TGLDSP)x4) Codon optimised

GACATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCTCTGTGGGAGACAGGGTGACTATCACCTGCAGGGC
CAGCCAGTGGATTGGCCCCGAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACCA
CGGCAGCATCCTGCAGTCCGGCGTGCCTAGCAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTCACCAT
CAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTATATGTACTACCCCCACACCTTCGGCCAG
GGCACCAAGGTGGAGATCAAGAGGGTGGAGCCTAAGTCTTCTGACAAGACCCACACCTGCCCCCCCTGCCCTGCC
CCAGAGCTGCTGGGAGGACCCAGCGTGTTCCTGTTCCCACCCAAGCCTAAGGACACCCTGATGATCAGCAGAACC

CCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGG
CGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGC
TGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACAAGGCCCTGCCTGCCC
CTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTGCCCCCTAGCAGA
GATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAG
TGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTC
CTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCTCCGTGATGCACGA
GGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGACCGGCCTCGACAGCCCCACTGG
CCTGGACAGCCCAACCGGACTGGATTCTCCCACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGAGCCCCAG
CAGCCTGAGCGCCAGCGTGGGGGACAGGGTGACTATCACCTGCAGGGCCTCCCAGTGGATTGGCCCCGAGCTGA
AGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACCACGGCAGCATCCTGCAGAGCGGCGTG
CCCTCAAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCCGAGGACTTC
GCCACCTACTACTGCCAGCAGTACATGTACTACCCCCACACCTTCGGCCAGGGCACCAAGGTGGAGATCAAAAGG

SEQ ID NO:144: DOM15-26-597 dAb N-((TGLDSP)x3) & C-terminal K-044-085 dAb ((TGLDSP)x4) Codon optimised

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCACGGGTCTGGACAGTCCGACTGGT
TTAGATTCACCTACGGGCTTGGACTCCCCAACCCACACCTGCCCCCCCTGCCCTGCCCCTGAGCTGCTGGGCGGAC
CCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAGGTGACCTGCGTGGT
GGTGGACGTGAGCCACGAGGACCCTGAGGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAACGCCA
AGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCATCAGC
AAGGCCAAGGGCCAGCCCAGGGAACCCCAGGTGTACACCCTGCCCCCCAGCCGGGACGAGCTGACCAAGAACCA
GGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGC
CCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACCGT
GGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACA
CCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGACCGGCCTCGACAGCCCCACTGGCCTGGACAGCCCAACCGGAC
TGGATTCTCCCACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCAGCGTGG
GGGACAGGGTGACTATCACCTGCAGGGCCTCCCAGTGGATTGGCCCCGAGCTGAAGTGGTATCAGCAGAAGCCC
GGCAAGGCCCCCAAGCTGCTGATCTACCACGGCAGCATCCTGCAGAGCGGCGTGCCCTCAAGGTTCTCAGGCAGC

GGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGT
ACATGTACTACCCCCACACCTTCGGCCAGGGCACCAAGGTGGAGATCAAAAGG

SEQ ID NO:145: DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb ((TGLDSP)x4) Codon optimised

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCTATGATGTGGGTGCGGCAGGCCCCTGGTAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCAAGCGGCAGCAACACCTACTACGCAGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACACTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCAGTGTACTACTGCGCCAAGGAC
CCACGGAAGCTGGACTACTGGGGTCAGGGCACCCTGGTGACCGTGAGCAGCGTGGAGCCTAAGTCTTCTGACAA
GACCCACACCTGCCCACCCTGCCCTGCCCCAGAGCTGCTGGGAGGACCCAGCGTGTTCCTGTTCCCACCCAAGCCT
AAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGA
GGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTAC
AACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTG
TAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCC
CCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGG
CTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACCCCCCC
TGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCA
ACGTGTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGG
CAAGACCGGCCTCGACAGCCCCACTGGCCTGGACAGCCCAACCGGACTGGATTCTCCCACCGGCCTGGACAGCCC
CGACATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCAGCGTGGGGGACAGGGTGACTATCACCTGCAGGG
CCTCCCAGTGGATTGGCCCCGAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACC
ACGGCAGCATCCTGCAGAGCGGCGTGCCCTCAAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTGACCA
TCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACATGTACTACCCCCACACCTTCGGCCA
GGGCACCAAGGTGGAGATCAAAAGG

SEQ ID NO:146: DMS1576 with C-terminal K-044-085 dAb ((TGLDSP)x4) Codon optimised

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCTAGCACCCACACCTGCCCCCCCT
GCCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAG

CCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCTGAGGTGAAGTTCAATTGGTACG
TGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGT
GTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGCCCT
GCCTGCCCCCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGGGAACCCCAGGTGTACACCCTGCCCCC
CAGCCGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGC
CGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCA
GCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGA
TGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGACCGGCCTCGACAGCC
CCACTGGCCTGGACAGCCCAACCGGACTGGATTCTCCCACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGA
GCCCCAGCAGCCTGAGCGCCAGCGTGGGGGACAGGGTGACTATCACCTGCAGGGCCTCCCAGTGGATTGGCCCC
GAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACCACGGCAGCATCCTGCAGAG
CGGCGTGCCCTCAAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCCGA
GGACTTCGCCACCTACTACTGCCAGCAGTACATGTACTACCCCCACACCTTCGGCCAGGGCACCAAGGTGGAGATC
AAAAGG

SEQ ID NO:147: DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb minus C-term R ((TGLDSP)x4) Codon optimised

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCTATGATGTGGGTGCGGCAGGCCCCTGGTAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCAAGCGGCAGCAACACCTACTACGCAGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACACTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCAGTGTACTACTGCGCCAAGGAC
CCACGGAAGCTGGACTACTGGGGTCAGGGCACCCTGGTGACCGTGAGCAGCGTGGAGCCTAAGTCTTCTGACAA
GACCCACACCTGCCCACCCTGCCCTGCCCCAGAGCTGCTGGGAGGACCCAGCGTGTTCCTGTTCCCACCCAAGCCT
AAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGA
GGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTAC
AACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTG
TAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCC
CCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGG
CTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACCCCCCC
TGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCA
ACGTGTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGG
CAAGACCGGCCTCGACAGCCCCACTGGCCTGGACAGCCCAACCGGACTGGATTCTCCCACCGGCCTGGACAGCCC
CGACATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCAGCGTGGGGGACAGGGTGACTATCACCTGCAGGG
CCTCCCAGTGGATTGGCCCCGAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACC

ACGGCAGCATCCTGCAGAGCGGCGTGCCCTCAAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTGACCA
TCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACATGTACTACCCCCACACCTTCGGCCA
GGGCACCAAGGTGGAGATCAAA

SEQ ID NO:148: DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb + A ((TGLDSP)x4) Codon optimised

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCTATGATGTGGGTGCGGCAGGCCCCTGGTAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCAAGCGGCAGCAACACCTACTACGCAGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACACTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCAGTGTACTACTGCGCCAAGGAC
CCACGGAAGCTGGACTACTGGGGTCAGGGCACCCTGGTGACCGTGAGCAGCGTGGAGCCTAAGTCTTCTGACAA
GACCCACACCTGCCCACCCTGCCCTGCCCCAGAGCTGCTGGGAGGACCCAGCGTGTTCCTGTTCCCACCCAAGCCT
AAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGA
GGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTAC
AACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTG
TAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCC
CCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGG
CTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACCCCCCC
TGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCA
ACGTGTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGG
CAAGACCGGCCTCGACAGCCCCACTGGCCTGGACAGCCCAACCGGACTGGATTCTCCCACCGGCCTGGACAGCCC
CGACATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCAGCGTGGGGGACAGGGTGACTATCACCTGCAGGG
CCTCCCAGTGGATTGGCCCCGAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACC
ACGGCAGCATCCTGCAGAGCGGCGTGCCCTCAAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTGACCA
TCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACATGTACTACCCCCACACCTTCGGCCA
GGGCACCAAGGTGGAGATCAAAAGGGCC

SEQ ID NO:149: DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb +AAA ((TGLDSP)x4) Codon optimised

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCTATGATGTGGGTGCGGCAGGCCCCTGGTAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCAAGCGGCAGCAACACCTACTACGCAGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACACTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCAGTGTACTACTGCGCCAAGGAC

CCACGGAAGCTGGACTACTGGGGTCAGGGCACCCTGGTGACCGTGAGCAGCGTGGAGCCTAAGTCTTCTGACAA
GACCCACACCTGCCCACCCTGCCCTGCCCCAGAGCTGCTGGGAGGACCCAGCGTGTTCCTGTTCCCACCCAAGCCT
AAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGA
GGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTAC
AACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTG
TAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCC
CCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGG
CTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACCCCCCC
TGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCA
ACGTGTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGG
CAAGACCGGCCTCGACAGCCCCACTGGCCTGGACAGCCCAACCGGACTGGATTCTCCCACCGGCCTGGACAGCCC
CGACATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCAGCGTGGGGGACAGGGTGACTATCACCTGCAGGG
CCTCCCAGTGGATTGGCCCCGAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACC
ACGGCAGCATCCTGCAGAGCGGCGTGCCCTCAAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTGACCA
TCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACATGTACTACCCCCACACCTTCGGCCA
GGGCACCAAGGTGGAGATCAAAAGGGCCGCCGCC

SEQ ID NO:150: DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb +T ((TGLDSP)x4) Codon optimised

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCTATGATGTGGGTGCGGCAGGCCCCTGGTAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCAAGCGGCAGCAACACCTACTACGCAGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACACTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCAGTGTACTACTGCGCCAAGGAC
CCACGGAAGCTGGACTACTGGGGTCAGGGCACCCTGGTGACCGTGAGCAGCGTGGAGCCTAAGTCTTCTGACAA
GACCCACACCTGCCCACCCTGCCCTGCCCCAGAGCTGCTGGGAGGACCCAGCGTGTTCCTGTTCCCACCCAAGCCT
AAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCCTGA
GGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTAC
AACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTG
TAAGGTGTCCAACAAGGCCCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCC
CCAGGTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGG
CTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACCCCCCC
TGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAGGGCA
ACGTGTTCAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGG
CAAGACCGGCCTCGACAGCCCCACTGGCCTGGACAGCCCAACCGGACTGGATTCTCCCACCGGCCTGGACAGCCC
CGACATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCAGCGTGGGGGACAGGGTGACTATCACCTGCAGGG

CCTCCCAGTGGATTGGCCCCGAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACC
ACGGCAGCATCCTGCAGAGCGGCGTGCCCTCAAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTGACCA
TCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACATGTACTACCCCCACACCTTCGGCCA
GGGCACCAAGGTGGAGATCAAAAGGACC

SEQ ID NO:151: DMS1576 with C-terminal K-044-085 dAb minus C-term R ((TGLDSP)x4) Codon optimised

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCTAGCACCCACACCTGCCCCCCCT
GCCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAG
CCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCTGAGGTGAAGTTCAATTGGTACG
TGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGT
GTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGCCCT
GCCTGCCCCCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGGGAACCCCAGGTGTACACCCTGCCCCC
CAGCCGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGC
CGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCA
GCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGA
TGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGACCGGCCTCGACAGCC
CCACTGGCCTGGACAGCCCAACCGGACTGGATTCTCCCACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGA
GCCCCAGCAGCCTGAGCGCCAGCGTGGGGGACAGGGTGACTATCACCTGCAGGGCCTCCCAGTGGATTGGCCCC
GAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACCACGGCAGCATCCTGCAGAG
CGGCGTGCCCTCAAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCCGA
GGACTTCGCCACCTACTACTGCCAGCAGTACATGTACTACCCCCACACCTTCGGCCAGGGCACCAAGGTGGAGATC
AAA

SEQ ID NO:152: DMS1576 with C-terminal K-044-085 dAb +A ((TGLDSP)x4) Codon optimised

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCTAGCACCCACACCTGCCCCCCCT

GCCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAG
CCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCTGAGGTGAAGTTCAATTGGTACG
TGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGT
GTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGCCCT
GCCTGCCCCCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGGGAACCCCAGGTGTACACCCTGCCCCC
CAGCCGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGC
CGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCA
GCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGA
TGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGACCGGCCTCGACAGCC
CCACTGGCCTGGACAGCCCAACCGGACTGGATTCTCCCACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGA
GCCCCAGCAGCCTGAGCGCCAGCGTGGGGGACAGGGTGACTATCACCTGCAGGGCCTCCCAGTGGATTGGCCCC
GAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACCACGGCAGCATCCTGCAGAG
CGGCGTGCCCTCAAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCCGA
GGACTTCGCCACCTACTACTGCCAGCAGTACATGTACTACCCCCACACCTTCGGCCAGGGCACCAAGGTGGAGATC
AAAAGGGCC

SEQ ID NO:153: DMS1576 with C-terminal K-044-085 dAb +AAA ((TGLDSP)x4) Codon optimised

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCTAGCACCCACACCTGCCCCCCCT
GCCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAG
CCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCTGAGGTGAAGTTCAATTGGTACG
TGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGT
GTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGCCCT
GCCTGCCCCCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGGGAACCCCAGGTGTACACCCTGCCCCC
CAGCCGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGC
CGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCA
GCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGA
TGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGACCGGCCTCGACAGCC
CCACTGGCCTGGACAGCCCAACCGGACTGGATTCTCCCACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGA
GCCCCAGCAGCCTGAGCGCCAGCGTGGGGGACAGGGTGACTATCACCTGCAGGGCCTCCCAGTGGATTGGCCCC
GAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACCACGGCAGCATCCTGCAGAG

CGGCGTGCCCTCAAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCCGA
GGACTTCGCCACCTACTACTGCCAGCAGTACATGTACTACCCCCACACCTTCGGCCAGGGCACCAAGGTGGAGATC
AAAAGGGCCGCCGCC

SEQ ID NO:154: DMS1576 with C-terminal K-044-085 dAb +T ((TGLDSP)x4) Codon optimised

GAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAG
CGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGA
GATCAGCCCCAGCGGCAGCAACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA
GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACC
CCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCTAGCACCCACACCTGCCCCCCCT
GCCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAG
CCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCTGAGGTGAAGTTCAATTGGTACG
TGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGT
GTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGCCCT
GCCTGCCCCCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGGGAACCCCAGGTGTACACCCTGCCCCC
CAGCCGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGC
CGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCA
GCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGA
TGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGACCGGCCTCGACAGCC
CCACTGGCCTGGACAGCCCAACCGGACTGGATTCTCCCACCGGCCTGGACAGCCCCGACATCCAGATGACCCAGA
GCCCCAGCAGCCTGAGCGCCAGCGTGGGGGACAGGGTGACTATCACCTGCAGGGCCTCCCAGTGGATTGGCCCC
GAGCTGAAGTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACCACGGCAGCATCCTGCAGAG
CGGCGTGCCCTCAAGGTTCTCAGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCCGA
GGACTTCGCCACCTACTACTGCCAGCAGTACATGTACTACCCCCACACCTTCGGCCAGGGCACCAAGGTGGAGATC
AAAAGGACC

SEQ ID NO:155: N-terminal Fc fusion of DOM15-26-597 dAb ((TGLDSP)x3)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSTGLDSPTGLDSPTGLDSPTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRNELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:156 : N-terminal Fc fusion of DOM15-26-597 dAb ((TGLDSP)x4)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSTGLDSPTGLDSPTGLDSPTGLDSPTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:157: N-terminal Fc fusion of DOM15-26-597 dAb ((TGLDSP)x3 T113P mutation Fc)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSTGLDSPTGLDSPTGLDSPTHPCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:158: N-terminal Fc fusion of DOM15-26-597 dAb ((TGLDSP)x4 T113P mutation Fc)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSTGLDSPTGLDSPTGLDSPTGLDSPTHPCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:159: N-terminal Fc fusion of DOM15-26-597 dAb (IgG1 Hinge)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSVEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:160: N-terminal Fc fusion of 15-26-597 dAb (IgG3 Hinge)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSELKTPLGDTTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:161: N-terminal Fc fusion of 15-26-597 dAb (TVAAPS)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSTVAAPSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:162: C-terminal Fc fusion of K-044-085 dAb ((TGLDSP)x4)

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSP
TGLDSPTGLDSPDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:163: C-terminal Fc fusion of K-044-085 dAb (Helical Linker)

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKEAAAKEAAAKE
AAAKELAAKEAAAKEAAAKEAAAKELAADIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYH
GSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:164: N-terminal Fc fusion of K-044-085 dAb (IgG1 Hinge)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQYMYYPHTFGQGTKVEIKRVEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:165: N-terminal Fc fusion of K-044-085 dAb (ASTHP linker)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQYMYYPHTFGQGTKVEIKRASTHPCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK

SEQ ID NO:166: N-terminal Fc fusion of K-044-085 dAb ((TGLDSP)x4)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQYMYYPHTFGQGTKVEIKRTGLDSPTGLDSPTGLDSPTGLDSPTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:167: N-terminal Fc fusion of K-044-085 dAb (TVAAPS)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQYMYYPHTFGQGTKVEIKRTVAAPSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK

SEQ ID NO:168: N-terminal Fc fusion of K-044-085 dAb ((TGLDSP)x3)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQYMYYPHTFGQGTKVEIKRTGLDSPTGLDSPTGLDSPTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:169: N-terminal Fc fusion of K-044-085 dAb (IgG3 Hinge)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQYMYYPHTFGQGTKVEIKRELKTPLGDTTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:170: N-terminal Fc fusion of K-044-085-AS-Fc (H112A)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQYMYYPHTFGQGTKVEIKRASTATCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK

SEQ ID NO:171: C-terminal Fc fusion of K-044-085 dAb ((TGLDSP)x3)

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSP
TGLDSPDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTI
SSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:172: C-terminal Fc fusion of K-044-085 dAb (Albumin Domain 1)

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKHKDDNPNLPRLV
RPEVDVMDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:173: C-terminal Fc fusion of K-044-085 dAb (Albumin Domain 2)

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKENDEMPADLPSL
AADFVESKDDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTD
FTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:174: C-terminal Fc fusion of K-044-085 dAb (Albumin Domain 3-TFHAD)

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKEVDETYVPKEFN
AETFDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISS
LQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:175: C-terminal Fc fusion of K-044-085 dAb ((Gly4Ser)x3)

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSG
GGGSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:176: C-terminal Fc fusion of K-044-085 dAb ((Gly4Ser)x4)

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSG
GGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:177: K-044-085 dAb N-(VEPKSSDK linker) & C-terminal ((TGLDSP)x4)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQYMYYPHTFGQGTKVEIKRVEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVTITCRASQWIGPEL
KWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:178: K-044-085 dAb N-(ASTHP linker) & C-terminal ((TGLDSP)x4)

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQYMYYPHTFGQGTKVEIKRASTHPCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVTITCRASQWIGPELKWYQ
QKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:179: DOM15-26-597 dAb N-((TGLDSP)x3) & C-terminal K-044-085 dAb ((TGLDSP)x4)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSTGLDSPTGLDSPTGLDSPTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSS
LSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQY
MYYPHTFGQGTKVEIKR

SEQ ID NO:180: DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb ((TGLDSP)x4)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSVEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVT
ITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQ
GTKVEIKR

SEQ ID NO:181: DMS1576 with C-terminal K-044-085 dAb ((TGLDSP)x4)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVTITCRAS
QWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEI
KR

SEQ ID NO:182: DOM15-26-597 dAb N-((TGLDSP)x4) & C-terminal K-044-085 dAb ((TGLDSP)x4)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSTGLDSPTGLDSPTGLDSPTGLDSPTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQ
MTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:183: DOM15-26-597 dAb N-((TGLDSP)x3 T113P mutation Fc) & C-terminal K-044-085 dAb ((TGLDSP)x4)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSTGLDSPTGLDSPTGLDSPTHPCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSS
LSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQY
MYYPHTFGQGTKVEIKR

SEQ ID NO:184: DOM15-26-597 dAb N-((TGLDSP)x4 T113P mutation Fc) & C-terminal K-044-085 dAb ((TGLDSP)x4)

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSTGLDSPTGLDSPTGLDSPTGLDSPTHPCPPCPAPELLGGPS

VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQ
MTQSPSSLSASVGDRVTITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYMYYPHTFGQGTKVEIKR

SEQ ID NO:185: DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb minus C-term R ((TGLDSP)x4) Codon optimised

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSVEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVT
ITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQ
GTKVEIK

SEQ ID NO:186: DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb + A ((TGLDSP)x4) Codon optimised

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSVEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVT
ITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQ
GTKVEIKRA

SEQ ID NO:187: DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb +AAA ((TGLDSP)x4) Codon optimised

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSVEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD

KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVT
ITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQ
GTKVEIKRAAA

SEQ ID NO:188: DOM15-26-597 dAb N-(VEPKSSDK linker) & C-terminal K-044-085 dAb +T ((TGLDSP)x4) Codon optimised

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSVEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVT
ITCRASQWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQ
GTKVEIKRT

SEQ ID NO:189: DMS1576 with C-terminal K-044-085 dAb minus C-term R ((TGLDSP)x4) Codon optimised

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVTITCRAS
QWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEI
K

SEQ ID NO:190: DMS1576 with C-terminal K-044-085 dAb +A ((TGLDSP)x4) Codon optimised

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVTITCRAS
QWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEI
KRA

SEQ ID NO:191: DMS1576 with C-terminal K-044-085 dAb +AAA ((TGLDSP)x4) Codon optimised

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVTITCRAS
QWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEI
KRAAA

SEQ ID NO:192: DMS1576 with C-terminal K-044-085 dAb +T ((TGLDSP)x4) Codon optimised

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSNTYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGKTGLDSPTGLDSPTGLDSPTGLDSPDIQMTQSPSSLSASVGDRVTITCRAS
QWIGPELKWYQQKPGKAPKLLIYHGSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMYYPHTFGQGTKVEI
KRT

Claims

1. An antigen binding construct comprising two epitope binding domains separated by a single chain Fc region of an antibody, wherein each epitope binding domain in capable of binding to VEGF.
2. An antigen binding construct according to claim 1, wherein each epitope binding domain is a domain antibody (dAb).
3. An antigen binding construct according to claim 2, wherein one dAb is attached to the N-terminus of the single chain Fc region and the other dAb is attached to the C-terminus of the single chain Fc region.
4. An antigen binding construct according to claim 3, wherein the dAb attached to the N-terminus of the single chain Fc region is a heavy chain dAb.
5. An antigen binding construct according to claim 3, wherein the dAb attached to the N-terminus of the single chain Fc region is a light chain dAb.
6. An antigen binding construct according to any of claims 3 to 5, wherein the dAb attached to the C-terminus of the single chain Fc region is a light chain dAb.
7. An antigen binding construct according to claim 5 or claim 6, wherein the light chain dAb is a kappa or lambda light chain dAb.
8. An antigen binding construct according to any of claims 2 to 7, wherein each dAb is independently selected and comprises an amino acid sequence which is at least 90% identical to the sequence of DOM 15-26-593, DOM 15-26-597, DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255.
9. An antigen binding construct according to any of claims 2 to 8, wherein each dAb is independently selected and comprises an amino acid sequence which is at least 97% identical to the sequence of DOM 15-26-593, DOM 15-26-597, DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255.
10. An antigen binding construct according to any of claims 2 to 9, wherein each dAb is independently selected from the group consisting of: DOM 15-26-593, DOM 15-26-597, DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 and DT02-K-044-255.
11. An antigen binding construct according to any of claims 3 to 10, wherein the dAb attached to the N-terminus of the single chain Fc region comprises an amino acid sequence which is at least 90% identical to the sequence of DOM 15-26-597 or DT02-K-044-085.
12. An antigen binding construct according to any of claims 3 to 11, wherein the dAb attached to the N-terminus of the single chain Fc region comprises an amino acid sequence which is at least 97% identical to the sequence of DOM 15-26-597 or DT02-K-044-085.
13. An antigen binding construct according to claim 11 or claim 12, wherein the dAb attached to the N-terminus of the single chain Fc region is DOM 15-26-597 or DT02-K-044-085.
14. An antigen binding construct according to any of claims 3 to 13, wherein the dAb attached to the C-terminus of the single chain Fc region comprises an amino acid sequence which is at least 90% identical

to the sequence of DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255.

15. An antigen binding construct according to any of claims 3 to 14, wherein the dAb attached to the C-terminus of the single chain Fc region comprises an amino acid sequence which is at least 97% identical to the sequence of DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255.

16. An antigen binding construct according to claim 14 or claim 15, wherein the dAb attached to the C-terminus of the single chain Fc region is DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255.

17. An antigen binding construct according to claim 16, wherein the dAb attached to the C-terminus of the single chain Fc region is DT02-K-044-085.

18. An antigen binding construct according to any of claims 2 to 7, wherein each dAb is independently selected and comprises:

viii) one or more of CDR sequences SEQ ID No. 111, 112 and 113;

ix) one or more of CDR sequences SEQ ID No. 114, 115 and 116;

x) one or more of CDR sequences SEQ ID No. 117, 118 and 119;

xi) one or more of CDR sequences SEQ ID No. 120, 121 and 122;

xii) one or more of CDR sequences SEQ ID No. 123, 124 and 125;

xiii) one or more of CDR sequences SEQ ID No. 126, 127 and 128; or

xiv) one or more of CDR sequences SEQ ID No. 129, 130 and 131.

19. An antigen binding protein according to claim 18, wherein the dAb attached to the N-terminus of the Fc region of an antibody comprises one or more CDR sequences SEQ ID No. 114, 115 and 116, and the dAb attached to the C-terminus of the Fc region of an antibody comprises one or more CDR sequences SEQ ID No. 117, 118 and 119.

20. An antigen binding construct comprising one epitope binding domain attached to a single chain Fc region of an antibody, wherein the epitope binding domain is a dAb which comprises an amino acid sequence which is at least 90% identical to the sequence of DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255, and further wherein the epitope binding domain is capable of binding to VEGF.

21. An antigen binding construct comprising one epitope binding domain attached to a single chain Fc region of an antibody, wherein the epitope binding domain is a dAb which comprises an amino acid sequence which is at least 97% identical to the sequence of DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255, and further wherein the epitope binding domain is capable of binding to VEGF.

22. An antigen binding construct according to claim 20 or claim 21, wherein the dAb is DT02-K-044-085, DT02-K-044-251, DT02-K-044-232, DT02-K-044-236 or DT02-K-044-255.

23. An antigen binding construct according to claim 20 or claim 21, wherein the dAb comprises:

vi) one or more of CDR sequences SEQ ID No. 117, 118 and 119;

vii) one or more of CDR sequences SEQ ID No. 120, 121 and 122;

viii) one or more of CDR sequences SEQ ID No. 123, 124 and 125;

ix) one or more of CDR sequences SEQ ID No. 126, 127 and 128; or

x) one or more of CDR sequences SEQ ID No. 129, 130 and 131.

24. An antigen binding construct according to any preceding claim, wherein the Fc region is an IgG1, IgG2, IgG3, IgG4, IgG4PE; or a fragment or variant thereof.

25. An antigen binding construct according to claim 24, wherein the Fc region comprises one or more of the CH1, CH2 and CH3 constant region antibody domains, and optionally a hinge region (i.e between CH1 and CH2).

26. An antigen binding construct according to claim 25, wherein the Fc region comprises the CH2 and CH3 constant region antibody domains.

27. An antigen binding construct according to claim 24, wherein the Fc region is the IgG1 sequence of SEQ ID No. 110.

28. An antigen binding construct according to any of the preceding claims, wherein one or both of the epitope binding domain(s) are attached to the Fc region through a linker.

29. An antigen binding construct according to claim 28, wherein each linker is independently selected and comprises 1 to 150 amino acids.

30. An antigen binding construct according to claim 29, wherein each linker is independently selected and comprises greater than 7 amino acids.

31. An antigen binding construct according to claim 28, wherein each linker is independently selected from the group consisting of IgG1 hinge, AS, Fibronectin x 3, Fibronectin x 4, and helical .

32. An antigen binding construct according to claim 31, wherein the linker through which an epitope binding domain attaches to the N-terminus of the Fc region is IgG1 hinge, AS or Fib-3.

33. An antigen binding construct according to claim 32, wherein the linker through which an epitope binding domain attaches to the N-terminus of the Fc region is IgG1 hinge or AS.

34. An antigen binding construct according to any of claims 31 to 33, wherein the linker through which an epitope binding domain attaches to the C-terminus of the Fc region is Fib-4 or a helical linker.

35. An antigen binding construct according to claim 34, wherein the linker through which an epitope binding domain attaches to the C-terminus of the Fc region is Fib-4.

36. An antigen binding construct according to claim 1, which is:

(a) DOM 15-26-597 – AS – Fc (SEQ ID No. 110) – Fibronectin x 4 – DT02-K-044-085; or

(b) DOM 15-26-597 – IgG1 hinge – Fc (SEQ ID No. 110) – Fibronectin x 4 – DT02-K-044-085; or

h) (c) A variant of (a) or b) having at least 97% amino acid identity across the whole sequence; or

i) (d) A variant of (a) or b), wherein each dAb sequence has at least 97% sequence identity to the sequence of the specific dAb.

37. An antigen binding construct according to any of claims 2 to 36, wherein one or more dAbs comprise:

a) a C-terminal sequence consisting of the sequence VTVS(S)nX [for a VH dAb] or VEIKpRqX [for a VL dAb]; and also optionally

b) one or more amino acid substitutions at positions 14, 41, 108, 110, or 112 compared to a human germline framework sequence;

wherein:

n represents an integer independently selected from 0 or 1;

p and q each represent 0 or 1 such that when p represents 1 q may be 0 or 1 and such that when p represents 0, q also represents 0;

X may be present or absent, and if present represents an amino acid extension of 1 to 8 amino acids residues;

with the further proviso that if X is absent.

38. An antigen binding construct according to claim 37, wherein for a VL dAb the C-terminal sequence is VEIKpRqX and p is 1, q is 0 and X is absent.

39. An antigen binding construct according to claim 37, wherein for a VL dAb the C-terminal sequence is VEIKpRqX and p is 1, q is 1 and X is A, AAA or T.

40. A dimer comprising two antigen binding constructs according to any of the preceding claims.

41. A dimer according to claim 40, which is a hetero- or homodimer.

42. A pharmaceutical composition comprising a dimer according to claim 40 or claim 41, and one or more pharmaceutically acceptable carrier(s).

43. A dimer according to claim 40 or claim 41, for use in medicine.

44. A dimer according to claim 40 or claim 41, for use in the treatment of diseases associated with VEGF signalling, such as cancer and/or ocular diseases such as Diabetic Macular Edema (DME), Wet AMD, Diabetic retinopathy, RVO, and Corneal neovascularisation.

45. Use of a dimer according to claim 40 or claim 41, for the manufacture of a medicament for treating diseases associated with VEGF signalling, such as cancer and/or ocular diseases such as Diabetic Macular Edema (DME), Wet AMD, Diabetic retinopathy, RVO, and Corneal neovascularisation.

46. A method of treating a patient suffering from diseases associated with VEGF signalling, such as cancer and ocular diseases such as Diabetic Macular Edema (DME), Wet AMD, Diabetic retinopathy, RVO and Corneal neovascularisation, comprising administering a therapeutic amount of a dimer according to claim 40 or claim 41.

47. A dimer for use according to any of claims 44 to 46 in the treatment or phophylaxis of a human patient.

48. A polynucleotide sequence encoding an antigen binding construct according to any one of claims 1- 39.

49. A recombinant transformed or transfected host cell comprising one or more polynucleotide sequences encoding an antigen binding construct according to any of claims 1-39.

50. A method for the production of an antigen binding construct according to claims 1-39, which method comprises the step of culturing a host cell of claim 39 and isolating the antigen binding construct.

51. An antigen binding construct produced by the method of claim 50.