DUAL VARIABLE DOMAIN IMMUNOGLOBULINS AND USES THEREOF

Cross-Reference to Related Applications

This application claims priority to U.S. Provisional Patent Application No. 61/174,711, filed May 1, 2009, which is hereby expressly incorporated herein by reference in its entirety for any purpose.

Field of the Invention

The present invention relates to multivalent and multispecific binding proteins, methods of making, and specifically to their uses in the, diagnosis, prevention and/or treatment of acute and chronic inflammatory diseases, cancer, and other diseases.

Background of the Invention

Engineered proteins, such as multispecific antibodies capable of binding two or more antigens are known in the art. Such multispecific binding proteins can be generated using cell fusion, chemical conjugation, or recombinant DNA techniques.

Bispecific antibodies have been produced using quadroma technology (see Milstein, C. and A.C. Cuello (1983) Nature 305(5934):537-40) based on the somatic fusion of two different hybridoma cell lines expressing murine monoclonal antibodies (mAbs) with the desired specificities of the bispecific antibody. Because of the random pairing of two different immunoglobulin (Ig) heavy and light chains within the resulting hybrid–hybridoma (or quadroma) cell line, up to ten different Ig species are generated, of which only one is the functional bispecific antibody. The presence of mis-paired by-products, and significantly reduced production yields, means sophisticated purification procedures are required.

Bispecific antibodies can also be produced by chemical conjugation of two different mAbs (see Staerz, U.D., et al. (1985) Nature 314(6012): 628-31). This approach does not yield homogeneous preparation. Other approaches have used chemical conjugation of two different mAbs or smaller antibody fragments (see Brennan, M., et al. (1985) Science 229(4708): 81-3).

Another method used to produce bispecific antibodies is the coupling of two parental antibodies with a hetero-bifunctional crosslinker, but the resulting bispecific antibodies suffer from significant molecular heterogeneity because reaction of the crosslinker with the parental antibodies is not site-directed. To obtain more homogeneous preparations of bispecific antibodies two different Fab fragments have been chemically crosslinked at their hinge cysteine residues in a site-directed manner (see Glennie, M.J., et al. (1987) J. Immunol. 139(7): 2367-75). But this method results in Fab'2 fragments, not full IgG molecule.

A wide variety of other recombinant bispecific antibody formats have been developed (see Kriangkum, J., et al. (2001) Biomol. Eng. 18(2): 31-40). Amongst them tandem single-chain Fv molecules and diabodies, and various derivatives thereof, are the most widely used. Routinely, construction of these molecules starts from two single-chain Fv (scFv) fragments that recognize different antigens (see Economides, A.N., et al. (2003) Nat. Med. 9(1): 47-52). Tandem scFv molecules (taFv) represent a straightforward format simply connecting the two scFv molecules with an additional peptide linker. The two scFv fragments present in these tandem scFv molecules form separate folding entities. Various linkers can be used to connect the two scFv fragments and linkers with a length of up to 63 residues (see Nakanishi, K., et al. (2001) Ann. Rev. Immunol. 19: 423-74). Although the parental scFv fragments can normally be expressed in soluble form in bacteria, it is, however, often observed that tandem scFv molecules form insoluble aggregates in bacteria. Hence, refolding protocols or the use of mammalian expression systems are routinely applied to produce soluble tandem scFv molecules. In a recent study, *in vivo* expression by transgenic rabbits and cattle of a tandem scFv directed against CD28 and a melanoma-associated proteoglycan was reported (see Gracie, J.A., et al. (1999) J. Clin. Invest. 104(10): 1393-401). In this construct, the two scFv molecules were connected by a CH1 linker and serum concentrations of up to 100 mg/L of the bispecific antibody were found. Various strategies including variations of the domain order or using middle linkers with varying length or flexibility were employed to allow soluble expression in bacteria. A few studies have now reported expression of soluble tandem scFv molecules in bacteria (see Leung, B.P., et al. (2000) J. Immunol. 164(12): 6495-502; Ito, A., et al. (2003) J. Immunol. 170(9): 4802-9; Karni, A., et al. (2002) J. Neuroimmunol. 125(1-2): 134-40) using either a very short Ala3 linker or long glycine/serine-rich linkers. In a recent study, phage display of a tandem scFv repertoire containing randomized middle linkers with a length of 3 or 6 residues was employed to enrich for those molecules that are produced in soluble and active form in bacteria. This approach resulted in the isolation of a tandem scFv molecule with a 6 amino acid residue linker (see Arndt, M. and J. Krauss (2003) Methods Mol. Biol. 207: 305-21). It is unclear whether this linker sequence represents a general solution to the soluble expression of tandem scFv molecules. Nevertheless, this study demonstrated that phage display of tandem scFv molecules in combination with directed mutagenesis is a powerful tool to enrich for these molecules, which can be expressed in bacteria in an active form.

Bispecific diabodies (Db) utilize the diabody format for expression. Diabodies are produced from scFv fragments by reducing the length of the linker connecting the VH and VL domain to approximately 5 residues (see Peipp, M. and T. Valerius (2002) Biochem. Soc. Trans. 30(4): 507-11). This reduction of linker size facilitates dimerization of two polypeptide chains by crossover pairing of the VH and VL domains. Bispecific diabodies are produced by expressing,

two polypeptide chains with, either the structure VHA-VLB and VHB-VLA (VH-VL configuration), or VLA-VHB and VLB-VHA (VL-VH configuration) within the same cell. A large variety of different bispecific diabodies have been produced in the past and most of them can be expressed in soluble form in bacteria. However, a recent comparative study demonstrates that the orientation of the variable domains can influence expression and formation of active binding sites (see Mack, M. et al.(1995) Proc. Natl. Acad. Sci. U S A 92(15): 7021-5). Nevertheless, soluble expression in bacteria represents an important advantage over tandem scFv molecules. However, since two different polypeptide chains are expressed within a single cell inactive homodimers can be produced together with active heterodimers. This necessitates the implementation of additional purification steps in order to obtain homogenous preparations of bispecific diabodies. One approach to force the generation of bispecific diabodies is the production of knob-into-hole diabodies (see Holliger, P., T. Prospero, and G. Winter (1993) Proc. Natl. Acad. Sci. U S A 90(14): 6444-8.18). This was demonstrated for a bispecific diabody directed against HER2 and CD3. A large knob was introduced in the VH domain by exchanging Val37 with Phe and Leu45 with Trp and a complementary hole was produced in the VL domain by mutating Phe98 to Met and Tyr87 to Ala, either in the anti- HER2 or the anti-CD3 variable domains. By using this approach the production of bispecific diabodies could be increased from 72% by the parental diabody to over 90% by the knob-into-hole diabody. Importantly, production yields did only slightly decrease as a result of these mutations. However, a reduction in antigen-binding activity was observed for several analyzed constructs. Thus, this rather elaborate approach requires the analysis of various constructs in order to identify those mutations that produce heterodimeric molecule with unaltered binding activity. In addition, such approach requires mutational modification of the immunoglobulin sequence at the constant region, thus creating non-native and non-natural form of the antibody sequence, which may result in increased immunogenicity, poor *in vivo* stability, as well as undesirable pharmacokinetics.

Single-chain diabodies (scDb) represent an alternative strategy to improve the formation of bispecific diabody-like molecules (see Holliger, P. and G. Winter (1997) Cancer Immunol. Immunother. 45(3-4): 128-30; Wu, A.M., et al. (1996) Immunotechnology 2(1): p. 21-36). Bispecific single-chain diabodies are produced by connecting the two diabody-forming polypeptide chains with an additional middle linker with a length of approximately 15 amino acid residues. Consequently, all molecules with a molecular weight corresponding to monomeric single-chain diabodies (50-60 kDa) are bispecific. Several studies have demonstrated that bispecific single chain diabodies are expressed in bacteria in soluble and active form with the majority of purified molecules present as monomers (see Holliger, P. and G. Winter (1997) Cancer Immunol. Immunother. 45(3-4): 128-30; Wu, A.M., et al. (1996) Immunotechnol. 2(1): 21-36; Pluckthun, A. and P. Pack (1997) Immunotechnol. 3(2): 83-105; Ridgway, J.B., et al.

(1996) Protein Engin. 9(7): 617-21). Thus, single-chain diabodies combine the advantages of tandem scFvs (all monomers are bispecific) and diabodies (soluble expression in bacteria).

More recently diabodies have been fused to Fc to generate more Ig-like molecules, named di-diabodies (see Lu, D., et al. (2004) J. Biol. Chem. 279(4): 2856-65). In addition, multivalent antibody construct comprising two Fab repeats in the heavy chain of an IgG and capable of binding four antigen molecules has been described (see WO 0177342A1, and Miller, K., et al. (2003) J. Immunol. 170(9): 4854-61).

There is a need in the art for improved multivalent binding proteins capable of binding two or more antigens. U.S. Patent Application Serial No. 11/507,050 provides a novel family of binding proteins capable of binding two or more antigens with high affinity, which are called dual variable domain immunoglobulins (DVD-Ig™). The present invention provides further novel binding proteins capable of binding two or more antigens.

Summary of the Invention

In a first aspect, there is provided a binding protein comprising first and second polypeptide chains, each independently comprising VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first variable domain;

VD2 is a second variable domain;

C is constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 is an Fc region;

n is 0 or 1,

wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site and the VD2 domains on the first and second polypeptide chains form a second functional target binding site, and wherein the binding protein is capable of binding:

a. EGFR and CD3, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,

three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or

three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328, and

the binding protein is capable of binding to EGFR with an EC50 of at most 157.62 nM as measured by direct binding ELISA and/or with an IC50 of at most 16 pM as measured by rCTL activity, and

the variable domains that form a functional target binding site for CD3 comprise three CDRs from SEQ ID NO: 27 and three CDRs from SEQ ID NO: 28;

b. EGFR and IGF1R, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,

three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or

three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328,

and the binding protein is capable of binding to EGFR with an EC50 of at most 286.41 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for IGFR1 comprise three CDRs from SEQ ID NO: 31 and three CDRs from SEQ ID NO: 32 and the binding protein is capable of binding to IGF1R with an EC50 of at most 78.58 nM as measured by direct binding ELISA;

c. EGFR and RON, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,

three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or

three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328,

and

the binding protein is capable of binding to EGFR with an EC50 of at most 29.93 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for RON comprise three CDRs from SEQ ID NO: 29 and three CDRs from SEQ ID NO: 30 and the binding protein is capable of binding to RON with an EC50 of at most 24.99 nM as measured by direct binding ELISA;

d. EGFR and HGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,

three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or

three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328,

and

the binding protein is capable of binding to EGFR with an EC50 of at most 332.05 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for HGF comprise three CDRs from SEQ ID NO: 33 and three CDRs from SEQ ID NO: 34 and the binding protein is capable of binding to HGF with an EC50 of at most 159.04 nM as measured by direct binding ELISA;

e. EGFR and VEGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,

three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or

three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328, and

the binding protein is capable of binding to EGFR with an EC50 of at most 561.59 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for VEGF comprise

three CDRs from SEQ ID NO: 35 and three CDRs from SEQ ID NO: 36,

three CDRs from SEQ ID NO: 53 and three CDRs from SEQ ID NO: 54, or

three CDRs from SEQ ID NO: 55 and three CDRs from SEQ ID NO: 56,

and the binding protein is capable of binding to VEGF with an EC50 of at most 528.26 nM as measured by direct binding ELISA;

f. EGFR and Erb33, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,

three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or

three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328, and

the binding protein is capable of binding to EGFR with an EC50 of at most 726.39 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for Erb33 comprise

three CDRs from SEQ ID NO: 45 and three CDRs from SEQ ID NO: 46,

three CDRs from SEQ ID NO: 47 and three CDRs from SEQ ID NO: 48, or

three CDRs from SEQ ID NO: 51 and three CDRs from SEQ ID NO: 52, and

the binding protein is capable of binding to Erb33 with an EC50 of at most 485.26 nM as measured by direct binding ELISA;

g. EGFR and DLL-4, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 39 and three CDRs from SEQ ID NO: 40,
three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,
three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,
three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or
three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328,
and

the binding protein is capable of binding to EGFR with an EC50 of at most 189.14 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for DLL-4 comprise three CDRs from SEQ ID NO: 37 and three CDRs from SEQ ID NO: 38 and the binding protein is capable of binding to DLL-4 with an EC50 of at most 4.01 nM as measured by direct binding ELISA;

h. EGFR and PLGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 39 and three CDRs from SEQ ID NO: 40,
three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,
three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,
three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or
three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328,
and

the binding protein is capable of binding to EGFR with an EC50 of at most 119.88 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for PLGF comprise three CDRs from SEQ ID NO: 41 and three CDRs from SEQ ID NO: 42 and the binding protein is capable of binding to PLGF with an EC50 of at most 0.74 nM as measured by direct binding ELISA;

or

i. EGFR and EGFR, wherein:

the variable domains that form functional target binding sites for EGFR each independently comprise

three CDRs from SEQ ID NO: 39 and three CDRs from SEQ ID NO: 40,
three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,
three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, and/or

three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328, and

the binding protein is capable of binding to EGFR with an EC50 of at most 6.83 nM as measured by direct binding ELISA and/or with a KD of at most 4.20E-08 M as measured by surface plasmon resonance.

In a second aspect, there is provided a binding protein comprising first and second polypeptide chains, each independently comprising VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first variable domain;

VD2 is a second variable domain;

C is constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 is an Fc region;

n is 0 or 1,

wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site and the VD2 domains on the first and second polypeptide chains form a second functional target binding site, and wherein the binding protein is capable of binding:

a. EGFR and CD3, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NOs: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 6.83 nM as measured by direct binding ELISA and/or with a KD of at most 4.20E-08 M as measured by surface plasmon resonance, and

the variable domains that form a functional target binding site for CD3 comprise SEQ ID NO: 27 and/or SEQ ID NO: 28;

b. EGFR and IGF1R, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NOs: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 286.41 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for IGFR1 comprise SEQ ID NO: 31 and/or SEQ ID NO: 32 and the binding protein is capable of binding to IGF1R with an EC50 of at most 78.58 nM as measured by direct binding ELISA;

c. EGFR and RON, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NOs: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 29.93 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for RON comprise SEQ ID NO: 29 and/or SEQ ID NO: 30 and the binding protein is capable of binding to RON with an EC50 of at most 24.99 nM as measured by direct binding ELISA;

d. EGFR and HGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NOs: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 332.05 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for HGF comprise SEQ ID NO: 33 and/or SEQ ID NO: 34 and the binding protein is capable of binding to HGF with an EC50 of at most 159.04 nM as measured by direct binding ELISA;

e. EGFR and VEGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NO: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 561.59 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for VEGF comprise a sequence selected from a group consisting of SEQ ID NO: 35, 36, and 53-56 and the binding protein is capable of binding to VEGF with an EC50 of at most 528.26 nM as measured by direct binding ELISA;

f. EGFR and Erb33, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NO: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 726.39 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for Erb33 comprise a sequence selected from a group consisting of SEQ ID NO: 45-48 and 51-52 and the binding protein is capable of binding to Erb33 with an EC50 of at most 485.26 nM as measured by direct binding ELISA;

g. EGFR and DLL-4, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NO: 39, 40, 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 189.14 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for DLL-4 comprise SEQ ID NO: 37 and/or SEQ ID NO: 38 and the binding protein is capable of binding to DLL-4 with an EC50 of at most 4.01 nM as measured by direct binding ELISA;

h. EGFR and PLGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NO: 39, 40, 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 119.88 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for PLGF comprise SEQ ID NO: 41 and/or SEQ ID NO: 42 and the binding protein is capable of binding to PLGF with an EC50 of at most 0.74 nM as measured by direct binding ELISA;

or

i. EGFR and EGFR, wherein:

the variable domains that form functional target binding sites for EGFR each independently comprise a sequence selected from a group consisting of SEQ ID NO: 39, 40, 49, 50, and 323-28 and is capable of binding to EGFR with an EC50 of at most 6.83 nM as measured by direct binding ELISA and/or with a KD of at most 4.20x10-08 M as measured by surface plasmon resonance.

In a third aspect, there is provided a binding protein as defined above, wherein the binding protein comprises variable domains that form functional binding sites for

a. EGFR and CD3, wherein the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for CD3 comprise SEQ ID NO: 27 and SEQ ID NO: 28;

b. EGFR and IGF1R, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for IGFR1 comprise SEQ ID NO: 31 and SEQ ID NO: 32;

c. EGFR and RON, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for RON comprise SEQ ID NO: 29 and SEQ ID NO: 30;

d. EGFR and HGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for HGF comprise SEQ ID NO: 33 and SEQ ID NO: 34;

e. EGFR and VEGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for VEGF comprise

SEQ ID NO: 35 and SEQ ID NO: 36,

SEQ ID NO: 53 and SEQ ID NO: 54, or

SEQ ID NO: 55 and SEQ ID NO: 56;

f. EGFR and Erb33, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for Erb33 comprise

SEQ ID NO: 45 and SEQ ID NO: 46,

SEQ ID NO: 47 and SEQ ID NO: 48, or

SEQ ID NO: 51 and SEQ ID NO: 52;

g. EGFR and DLL-4, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 39 and SEQ ID NO: 40,

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for DLL-4 comprise

SEQ ID NO: 37 and SEQ ID NO: 38;

h. EGFR and PLGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 39 and SEQ ID NO: 40,

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for PLGF comprise

SEQ ID NO: 41 and SEQ ID NO: 42;

or

i. EGFR and EGFR, wherein:

the variable domains that form functional target binding sites for EGFR each independently comprise

SEQ ID NO: 39 and SEQ ID NO: 40,

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328.

In a fourth aspect, there is provided a binding protein conjugate comprising a binding protein as defined above, the binding protein conjugate further comprising an immunoadhesion molecule, an imaging agent, a therapeutic agent, or a cytotoxic agent.

In a fifth aspect, there is provided a pharmaceutical composition comprising the binding protein as defined above or the binding protein conjugate as defined above, and a pharmaceutically acceptable carrier,
optionally comprising at least one additional therapeutic agent selected from the group consisting of an imaging agent, a cytotoxic agent, an angiogenesis inhibitor, a kinase inhibitor, a co-stimulation molecule blocker, an adhesion molecule blocker, an anti-cytokine antibody or functional fragment thereof, methotrexate, cyclosporin, rapamycin, FK506, a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NTHE), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteriod, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, and a cytokine antagonist.

In a sixth aspect, there is provided the use of the binding protein as defined above or the binding protein conjugate as defined above in the manufacture of a medicament for treating a disease or disorder.

In a seventh aspect, there is provided an isolated nucleic acid encoding a binding protein as defined above.

In an eighth aspect, there is provided a vector comprising an isolated nucleic acid as defined above, wherein the vector is
optionally selected from the group consisting of pcDNA, pTT, pTT3, pEFBOS, pBV, pJV, pcDNA3.1 TOPO, pEF6 TOPO, pHybE and pBJ.

In a ninth aspect, there is provided a host cell comprising the vector as defined above, wherein the host is optionally
selected from the group consisting of a prokaryotic cell, an E.Coli, a eukaryotic cell, a protist cell, animal cell, plant cell, fungal cell, a mammalian cell, a yeast cell, an avian cell, an insect cell, a CHO cell, a COS cell, a S. cerevisiae cell, and an Sf9 cell.

In a tenth aspect, there is provided a method of producing a binding protein, comprising culturing the host cell as defined above in culture medium under conditions sufficient to produce the binding protein.

This disclosure pertains to multivalent binding proteins capable of binding two or more antigens. The present disclosure provides a novel family of binding proteins capable of binding two or more antigens with high affinity.

In one embodiment the disclosure provides a binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first variable domain, VD2 is a second variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1. In an embodiment the VD1 and VD2 in the binding protein are heavy chain variable domains. In another embodiment, the heavy chain variable domain is selected from the group consisting of a murine heavy chain variable domain, a human heavy chain variable domain, a CDR grafted heavy chain variable domain, and a humanized heavy chain variable domain. In yet another, embodiment VD1 and VD2 are capable of binding the same antigen. In another embodiment VD1 and VD2 are capable of binding different antigens. In still another embodiment, C is a heavy chain constant domain. For example, X1 is a linker with the proviso that X1 is not CH1. For example, X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6); RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAAA$(G_4S)_4$ (SEQ ID NO: 9); SAKTTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15); QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP (SEQ

ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22), GGGGSGGGGSGGGGS (SEQ ID NO: 23); GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25); GHEAAAVMQVQYPAS (SEQ ID NO: 26). In an embodiment, X2 is an Fc region. In another embodiment, X2 is a variant Fc region.

In an embodiment the binding protein disclosed herein comprises a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region.

In an embodiment, VD1 and VD2 in the binding protein are light chain variable domains. In an embodiment, the light chain variable domain is selected from the group consisting of a murine light chain variable domain, a human light chain variable domain, a CDR grafted light chain variable domain, and a humanized light chain variable domain. In one embodiment VD1 and VD2 are capable of binding the same antigen. In another embodiment VD1 and VD2 are capable of binding different antigens. In an embodiment, C is a light chain constant domain. In another embodiment, X1 is a linker with the proviso that X1 is not CL1. In an embodiment, X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6); RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAAA$(G_4S)_4$ (SEQ ID NO: 9); SAKTTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15); QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP (SEQ ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22), GGGGSGGGGSGGGGS (SEQ ID NO: 23); GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25); GHEAAAVMQVQYPAS (SEQ ID NO: 26). In an embodiment, the binding protein does not comprise X2.

In an embodiment, both the variable heavy and variable light chain comprise the same linker. In another embodiment, the variable heavy and variable light chain comprise different linkers. In another embodiment, both the variable heavy and variable light chain comprise a short (about 6 amino acids) linker. In another embodiment, both the variable heavy and variable light chain comprise a long (greater than 6 amino acids) linker. In another embodiment, the variable heavy chain comprises a short linker and the variable light chain comprises a long linker. In another embodiment, the variable heavy chain comprises a long linker and the variable light chain comprises a short linker.

In an embodiment the binding protein disclosed herein comprises a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region.

In another embodiment the disclosure provides a binding protein comprising two polypeptide chains, wherein said first polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region; and said second polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region. In a particular embodiment, the Dual Variable Domain (DVD) binding protein comprises four polypeptide chains wherein the first two polypeptide chains comprises VD1-(X1)n-VD2-C-(X2)n, respectively wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region; and the second two polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n respectively, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region. Such a Dual Variable Domain (DVD) protein has four antigen binding sites.

In another embodiment the binding proteins disclosed herein are capable of binding one or more targets. In an embodiment, the target is selected from the group consisting of cytokines, cell surface proteins, enzymes and receptors. In another embodiment, the binding protein is capable of modulating a biological function of one or more targets. In another embodiment, the binding protein is capable of neutralizing one or more targets. The binding protein of the disclosure is capable of binding cytokines selected from the group consisting of lymphokines, monokines, polypeptide hormones, receptors, or tumor markers. For example, the DVD-Ig of the disclosure is capable of binding two or more of the following: CD-3, RON, IGF1R, HGF, VEGF, DLL-4, EGFR, PLGF, ErbB3 RGMa, and tetanus toxoid (see also Table 2). In a specific embodiment the binding protein is capable of binding pairs of targets selected from the group consisting of.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 59 and SEQ ID NO. 61; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 60 and SEQ ID NO. 62. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) comprises a DVD heavy chain amino acid

sequence of SEQ ID NO. 59 and a DVD light chain amino acid sequence of SEQ ID NO: 60. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 61 and a DVD light chain amino acid sequence of SEQ ID NO: 62.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 63 and SEQ ID NO. 65; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 64 and SEQ ID NO. 66. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 63 and a DVD light chain amino acid sequence of SEQ ID NO: 64. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 65 and a DVD light chain amino acid sequence of SEQ ID NO: 66.

In third embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 67 and SEQ ID NO. 69; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 68 and SEQ ID NO. 70. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 67 and a DVD light chain amino acid sequence of SEQ ID NO: 68. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 69 and a DVD light chain amino acid sequence of SEQ ID NO: 70.

In fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 71 and SEQ ID NO. 73; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 72 and SEQ ID NO. 74. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 71 and a DVD light chain amino acid sequence of SEQ ID NO: 72. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and EGFR (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 73 and a DVD light chain amino acid sequence of SEQ ID NO: 74.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and RON comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 75 and SEQ ID NO. 77; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 76 and SEQ ID NO. 78. In an embodiment, the binding

protein capable of binding EGFR (seq. 2) and RON comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 75 and a DVD light chain amino acid sequence of SEQ ID NO: 76. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and RON has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 77 and a DVD light chain amino acid sequence of SEQ ID NO: 78.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and RON comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 79 and SEQ ID NO. 81; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 80 and SEQ ID NO. 82. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and RON comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 79 and a DVD light chain amino acid sequence of SEQ ID NO: 80. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and RON has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 81 and a DVD light chain amino acid sequence of SEQ ID NO: 82.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and RON comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 83 and SEQ ID NO. 85; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 84 and SEQ ID NO. 86. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and RON comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 83 and a DVD light chain amino acid sequence of SEQ ID NO: 84. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and RON has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 85 and a DVD light chain amino acid sequence of SEQ ID NO: 86.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and RON comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 87 and SEQ ID NO. 89; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 88 and SEQ ID NO. 90. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and RON comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 87 and a DVD light chain amino acid sequence of SEQ ID NO: 88. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and RON has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 89 and a DVD light chain amino acid sequence of SEQ ID NO: 90.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 91 and SEQ ID NO. 93; and a DVD light chain amino acid sequence selected from the

group consisting of SEQ ID NO. 92 and SEQ ID NO. 94. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 91 and a DVD light chain amino acid sequence of SEQ ID NO: 92. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 93 and a DVD light chain amino acid sequence of SEQ ID NO: 94.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 95 and SEQ ID NO. 97; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 96 and SEQ ID NO. 98. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 95 and a DVD light chain amino acid sequence of SEQ ID NO: 96. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 97 and a DVD light chain amino acid sequence of SEQ ID NO: 98.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 99 and SEQ ID NO. 101; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 100 and SEQ ID NO. 102. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 99 and a DVD light chain amino acid sequence of SEQ ID NO: 100. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 101 and a DVD light chain amino acid sequence of SEQ ID NO: 102.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 103 and SEQ ID NO. 105; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 104 and SEQ ID NO. 106. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 103 and a DVD light chain amino acid sequence of SEQ ID NO: 104. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 105 and a DVD light chain amino acid sequence of SEQ ID NO: 106.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ

ID NO. 107 and SEQ ID NO. 109; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 108 and SEQ ID NO. 110. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 107 and a DVD light chain amino acid sequence of SEQ ID NO: 108. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 109 and a DVD light chain amino acid sequence of SEQ ID NO: 110.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 111 and SEQ ID NO. 113; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 112 and SEQ ID NO. 114. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 111 and a DVD light chain amino acid sequence of SEQ ID NO: 112. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 113 and a DVD light chain amino acid sequence of SEQ ID NO: 114.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 115 and SEQ ID NO. 117; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 116 and SEQ ID NO. 118. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 115 and a DVD light chain amino acid sequence of SEQ ID NO: 116. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 117 and a DVD light chain amino acid sequence of SEQ ID NO: 118.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 119 and SEQ ID NO. 121; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 120 and SEQ ID NO. 122. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 119 and a DVD light chain amino acid sequence of SEQ ID NO: 120. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 121 and a DVD light chain amino acid sequence of SEQ ID NO: 122.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 123 and SEQ ID NO. 125; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 124 and SEQ ID NO. 126. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 123 and a DVD light chain amino acid sequence of SEQ ID NO: 124. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 125 and a DVD light chain amino acid sequence of SEQ ID NO: 126.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 127 and SEQ ID NO. 129; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 128 and SEQ ID NO. 130. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 127 and a DVD light chain amino acid sequence of SEQ ID NO: 128. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 129 and a DVD light chain amino acid sequence of SEQ ID NO: 130.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 131 and SEQ ID NO. 133; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 132 and SEQ ID NO. 134. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 131 and a DVD light chain amino acid sequence of SEQ ID NO: 132. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 133 and a DVD light chain amino acid sequence of SEQ ID NO: 134.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 135 and SEQ ID NO. 137; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 136 and SEQ ID NO. 138. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 135 and a DVD light chain amino acid sequence of SEQ ID NO: 136. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and CD3 has

a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 137 and a DVD light chain amino acid sequence of SEQ ID NO: 138.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 139 and SEQ ID NO. 141; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 140 and SEQ ID NO. 142. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 139 and a DVD light chain amino acid sequence of SEQ ID NO: 140. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 141 and a DVD light chain amino acid sequence of SEQ ID NO: 142.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 143 and SEQ ID NO. 145; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 144 and SEQ ID NO. 146. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 143 and a DVD light chain amino acid sequence of SEQ ID NO: 144. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 145 and a DVD light chain amino acid sequence of SEQ ID NO: 146.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 147 and SEQ ID NO. 149; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 148 and SEQ ID NO. 150. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 147 and a DVD light chain amino acid sequence of SEQ ID NO: 148. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 149 and a DVD light chain amino acid sequence of SEQ ID NO: 150.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 151 and SEQ ID NO. 153; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 152 and SEQ ID NO. 154. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 151 and a DVD light chain amino acid sequence of SEQ ID

NO: 152. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and IGF1R has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 153 and a DVD light chain amino acid sequence of SEQ ID NO: 154.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 155 and SEQ ID NO. 157; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 156 and SEQ ID NO. 158. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 155 and a DVD light chain amino acid sequence of SEQ ID NO: 156. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 157 and a DVD light chain amino acid sequence of SEQ ID NO: 158.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 159 and SEQ ID NO. 161; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 160 and SEQ ID NO. 162. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 159 and a DVD light chain amino acid sequence of SEQ ID NO: 160. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 161 and a DVD light chain amino acid sequence of SEQ ID NO: 162.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 163 and SEQ ID NO. 165; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 164 and SEQ ID NO. 166. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 163 and a DVD light chain amino acid sequence of SEQ ID NO: 164. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 165 and a DVD light chain amino acid sequence of SEQ ID NO: 166.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 167 and SEQ ID NO. 169; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 168 and SEQ ID NO. 170. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF comprises a DVD heavy chain amino

acid sequence of SEQ ID NO. 167 and a DVD light chain amino acid sequence of SEQ ID NO: 168. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and HGF has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 169 and a DVD light chain amino acid sequence of SEQ ID NO: 170.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 171 and SEQ ID NO. 173; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 172 and SEQ ID NO. 174. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 171 and a DVD light chain amino acid sequence of SEQ ID NO: 172. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 173 and a DVD light chain amino acid sequence of SEQ ID NO: 174.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 175 and SEQ ID NO. 177; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 176 and SEQ ID NO. 178. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 175 and a DVD light chain amino acid sequence of SEQ ID NO: 176. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 177 and a DVD light chain amino acid sequence of SEQ ID NO: 178.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 179 and SEQ ID NO. 181; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 180 and SEQ ID NO. 182. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 179 and a DVD light chain amino acid sequence of SEQ ID NO: 180. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 181 and a DVD light chain amino acid sequence of SEQ ID NO: 182.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 183 and SEQ ID NO. 185; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 184 and SEQ ID NO. 186. In an embodiment, the

binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 183 and a DVD light chain amino acid sequence of SEQ ID NO: 184. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 185 and a DVD light chain amino acid sequence of SEQ ID NO: 186.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 187 and SEQ ID NO. 189; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 188 and SEQ ID NO. 190. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 187 and a DVD light chain amino acid sequence of SEQ ID NO: 188. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 189 and a DVD light chain amino acid sequence of SEQ ID NO: 190.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 191 and SEQ ID NO. 193; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 192 and SEQ ID NO. 194. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 191 and a DVD light chain amino acid sequence of SEQ ID NO: 192. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 193 and a DVD light chain amino acid sequence of SEQ ID NO: 194.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 195 and SEQ ID NO. 197; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 196 and SEQ ID NO. 198. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 195 and a DVD light chain amino acid sequence of SEQ ID NO: 196. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 197 and a DVD light chain amino acid sequence of SEQ ID NO: 198.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 199 and SEQ ID NO. 201; and a DVD light chain amino acid sequence selected from the

group consisting of SEQ ID NO. 200 and SEQ ID NO. 202. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 199 and a DVD light chain amino acid sequence of SEQ ID NO: 200. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and DLL-4 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 201 and a DVD light chain amino acid sequence of SEQ ID NO: 202.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 203 and SEQ ID NO. 205; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 204 and SEQ ID NO. 206. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 203 and a DVD light chain amino acid sequence of SEQ ID NO: 204. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 205 and a DVD light chain amino acid sequence of SEQ ID NO: 206.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 207 and SEQ ID NO. 209; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 208 and SEQ ID NO. 210. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 207 and a DVD light chain amino acid sequence of SEQ ID NO: 208. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 209 and a DVD light chain amino acid sequence of SEQ ID NO: 210.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 211 and SEQ ID NO. 213; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 212 and SEQ ID NO. 214. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 211 and a DVD light chain amino acid sequence of SEQ ID NO: 212. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 213 and a DVD light chain amino acid sequence of SEQ ID NO: 214.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID

NO. 215 and SEQ ID NO. 217; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 216 and SEQ ID NO. 218. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 215 and a DVD light chain amino acid sequence of SEQ ID NO: 216. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and PLGF has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 217 and a DVD light chain amino acid sequence of SEQ ID NO: 218.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 219 and SEQ ID NO. 221; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 220 and SEQ ID NO. 222. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 219 and a DVD light chain amino acid sequence of SEQ ID NO: 220. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 221 and a DVD light chain amino acid sequence of SEQ ID NO: 222.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 223 and SEQ ID NO. 225; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 224 and SEQ ID NO. 226. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 223 and a DVD light chain amino acid sequence of SEQ ID NO: 224. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 225 and a DVD light chain amino acid sequence of SEQ ID NO: 226.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 227 and SEQ ID NO. 229; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 228 and SEQ ID NO. 230. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 227 and a DVD light chain amino acid sequence of SEQ ID NO: 228. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 229 and a DVD light chain amino acid sequence of SEQ ID NO: 230.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 231 and SEQ ID NO. 233; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 232 and SEQ ID NO. 234. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 231 and a DVD light chain amino acid sequence of SEQ ID NO: 232. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 3) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 233 and a DVD light chain amino acid sequence of SEQ ID NO: 234.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 235 and SEQ ID NO. 237; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 236 and SEQ ID NO. 238. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 235 and a DVD light chain amino acid sequence of SEQ ID NO: 236. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 237 and a DVD light chain amino acid sequence of SEQ ID NO: 238.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 239 and SEQ ID NO. 241; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 240 and SEQ ID NO. 242. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 239 and a DVD light chain amino acid sequence of SEQ ID NO: 240. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 241 and a DVD light chain amino acid sequence of SEQ ID NO: 242.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 243 and SEQ ID NO. 245; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 244 and SEQ ID NO. 246. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 243 and a DVD light chain amino acid sequence of SEQ ID NO: 244. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 245 and a DVD light chain amino acid sequence of SEQ ID NO: 246.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 247 and SEQ ID NO. 249; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 248 and SEQ ID NO. 250. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 247 and a DVD light chain amino acid sequence of SEQ ID NO: 248. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 249 and a DVD light chain amino acid sequence of SEQ ID NO: 250.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 251 and SEQ ID NO. 253; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 252 and SEQ ID NO. 254. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 251 and a DVD light chain amino acid sequence of SEQ ID NO: 252. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 253 and a DVD light chain amino acid sequence of SEQ ID NO: 254.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 255 and SEQ ID NO. 257; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 256 and SEQ ID NO. 258. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 255 and a DVD light chain amino acid sequence of SEQ ID NO: 256. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 257 and a DVD light chain amino acid sequence of SEQ ID NO: 258.

In a third embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 259 and SEQ ID NO. 261; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 260 and SEQ ID NO. 262. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 259 and a DVD light chain amino acid sequence of SEQ ID NO: 260. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 261 and a DVD light chain amino acid sequence of SEQ ID NO: 262.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 263 and SEQ ID NO. 265; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 264 and SEQ ID NO. 266. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 263 and a DVD light chain amino acid sequence of SEQ ID NO: 264. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and VEGF (seq. 3) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 265 and a DVD light chain amino acid sequence of SEQ ID NO: 266.

In an embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 267 and SEQ ID NO. 269; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 268 and SEQ ID NO. 270. In an embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 267 and a DVD light chain amino acid sequence of SEQ ID NO: 268. In another embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 269 and a DVD light chain amino acid sequence of SEQ ID NO: 270.

In a second embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 271 and SEQ ID NO. 273; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 272 and SEQ ID NO. 274. In an embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 271 and a DVD light chain amino acid sequence of SEQ ID NO: 272. In another embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 273 and a DVD light chain amino acid sequence of SEQ ID NO: 274.

In a third embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 275 and SEQ ID NO. 277; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 276 and SEQ ID NO. 278. In an embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 275 and a DVD light chain amino acid sequence of SEQ ID NO: 276. In another embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 277 and a DVD light chain amino acid sequence of SEQ ID NO: 278.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 279 and SEQ ID NO. 281; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 280 and SEQ ID NO. 282. In an embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 279 and a DVD light chain amino acid sequence of SEQ ID NO: 280. In another embodiment, the binding protein capable of binding EGFR (seq. 1) and RGMa has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 281 and a DVD light chain amino acid sequence of SEQ ID NO: 282.

In an embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 283 and SEQ ID NO. 285; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 284 and SEQ ID NO. 286. In an embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 283 and a DVD light chain amino acid sequence of SEQ ID NO: 284. In another embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 285 and a DVD light chain amino acid sequence of SEQ ID NO: 286.

In a second embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 287 and SEQ ID NO. 289; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 288 and SEQ ID NO. 290. In an embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 287 and a DVD light chain amino acid sequence of SEQ ID NO: 288. In another embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 289 and a DVD light chain amino acid sequence of SEQ ID NO: 290.

In a third embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 291 and SEQ ID NO. 293; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 292 and SEQ ID NO. 294. In an embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 291 and a DVD light chain amino acid sequence of SEQ ID NO: 292. In another embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 293 and a DVD light chain amino acid sequence of SEQ ID NO: 294.

In a fourth embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 295 and SEQ ID NO. 297; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 296 and SEQ ID NO. 298. In an embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 295 and a DVD light chain amino acid sequence of SEQ ID NO: 296. In another embodiment, the binding protein capable of binding EGFR (seq. 1) and tetanus toxoid has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 297 and a DVD light chain amino acid sequence of SEQ ID NO: 298.

In an embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 299 and SEQ ID NO. 301; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 300 and SEQ ID NO. 302. In an embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 299 and a DVD light chain amino acid sequence of SEQ ID NO: 300. In another embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 301 and a DVD light chain amino acid sequence of SEQ ID NO: 302.

In a second embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 303 and SEQ ID NO. 305; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 304 and SEQ ID NO. 306. In an embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 303 and a DVD light chain amino acid sequence of SEQ ID NO: 304. In another embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 305 and a DVD light chain amino acid sequence of SEQ ID NO: 306.

In a third embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 307 and SEQ ID NO. 309; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 308 and SEQ ID NO. 310. In an embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 307 and a DVD light chain amino acid sequence of SEQ ID NO: 308. In another embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 309 and a DVD light chain amino acid sequence of SEQ ID NO: 310.

In a fourth embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 311 and SEQ ID NO. 313; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 312 and SEQ ID NO. 314. In an embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 311 and a DVD light chain amino acid sequence of SEQ ID NO: 312. In another embodiment, the binding protein capable of binding VEGF (seq. 1) and tetanus toxoid has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 313 and a DVD light chain amino acid sequence of SEQ ID NO: 314.

In an embodiment, the binding protein capable of binding tetanus toxoid and tetanus toxoid comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 315 and SEQ ID NO. 317; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 316 and SEQ ID NO. 318. In an embodiment, the binding protein capable of binding tetanus toxoid and tetanus toxoid comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 315 and a DVD light chain amino acid sequence of SEQ ID NO: 316. In another embodiment, the binding protein capable of binding tetanus toxoid and tetanus toxoid has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 317 and a DVD light chain amino acid sequence of SEQ ID NO: 318.

In a second embodiment, the binding protein capable of binding tetanus toxoid and tetanus toxoid comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 319 and SEQ ID NO. 321; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 320 and SEQ ID NO. 322. In an embodiment, the binding protein capable of binding tetanus toxoid and tetanus toxoid comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 319 and a DVD light chain amino acid sequence of SEQ ID NO: 320. In another embodiment, the binding protein capable of binding tetanus toxoid and tetanus toxoid has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 321 and a DVD light chain amino acid sequence of SEQ ID NO: 322.

In an embodiment, the EGFR VH sequence of any of the above described DVD-Ig comprises the amino acid sequence of any one of SEQ ID NOs: 323, 325, or 327. In another embodiment, the EGFR VL sequence of any of the above described DVD-Ig comprises the amino acid sequence of any one of SEQ ID NOs: 324, 326, or 328.

In another embodiment the disclosure provides a binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein; VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from a second parent antibody or

antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n is an Fc region, wherein said (X2)n is either present or absent. In an embodiment, the Fc region is absent from the binding protein.

In another embodiment, the disclosure provides a binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein, VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein said (X2)n is either present or absent. In an embodiment, (X2)n is absent from the binding protein.

In another embodiment the binding protein of the disclosure comprises first and second polypeptide chains, wherein said first polypeptide chain comprises a first VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n is an Fc region, wherein said (X2)n is either present or absent; and wherein said second polypeptide chain comprises a second VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein said (X2)n is either present or absent. In another embodiment, the binding protein comprises two first polypeptide chains and two second polypeptide chains. In yet another embodiment, (X2)n is absent from the second polypeptide. In still another embodiment, the Fc region, if present in the first polypeptide is selected from the group consisting of native sequence Fc region and a variant sequence Fc region. In still another embodiment, the Fc region is selected from the group consisting of an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD.

In another embodiment the binding protein of the disclosure is a DVD-Ig capable of binding two antigens comprising four polypeptide chains, wherein, first and third polypeptide chains comprise VD1-(X1)n-VD2-C-(X2)n, wherein,VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n is an Fc region, wherein said (X2)n is either present or absent; and wherein second and fourth polypeptide chains comprise VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein said (X2)n is either present or absent.

The disclosure provides a method of making a DVD-Ig binding protein by preselecting the parent antibodies. In an embodiment, the method of making a Dual Variable Domain Immunoglobulin capable of binding two antigens comprising the steps of a) obtaining a first parent antibody or antigen binding portion thereof, capable of binding a first antigen; b) obtaining a second parent antibody or antigen binding portion thereof, capable of binding a second antigen; c) constructing first and third polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein, VD1 is a first heavy chain variable domain obtained from said first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from said second parent antibody or antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n is an Fc region, wherein said (X2)n is either present or absent; d) constructing second and fourth polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein, VD1 is a first light chain variable domain obtained from said first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from said second parent antibody or antigen binding thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein said (X2)n is either present or absent; e) expressing said first, second, third and fourth polypeptide chains; such that a Dual Variable Domain Immunoglobulin capable of binding said first and said second antigen is generated.

In still another embodiment, the disclosure provides a method of generating a Dual Variable Domain Immunoglobulin capable of binding two antigens with desired properties comprising the steps of a) obtaining a first parent antibody or antigen binding portion thereof, capable of binding a first antigen and possessing at least one desired property exhibited by the Dual Variable Domain Immunoglobulin; b) obtaining a second parent antibody or antigen binding portion thereof, capable of binding a second antigen and possessing at least one desired property exhibited by the Dual Variable Domain Immunoglobulin; c) constructing first and third polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein; VD1 is a first heavy chain variable domain obtained from said first parent antibody or antigen binding portion thereof; VD2

is a second heavy chain variable domain obtained from said second parent antibody or antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n is an Fc region, wherein said (X2)n is either present or absent; d) constructing second and fourth polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein; VD1 is a first light chain variable domain obtained from said first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from said second parent antibody or antigen binding portion thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein said (X2)n is either present or absent; e) expressing said first, second, third and fourth polypeptide chains; such that a Dual Variable Domain Immunoglobulin capable of binding said first and said second antigen with desired properties is generated.

In one embodiment, the VDI of the first and second polypeptide chains disclosed herein are obtained from the same parent antibody or antigen binding portion thereof. In another embodiment, the VDI of the first and second polypeptide chains disclosed herein are obtained from different parent antibodies or antigen binding portions thereof. In another embodiment, the VD2 of the first and second polypeptide chains disclosed herein are obtained from the same parent antibody or antigen binding portion thereof. In another embodiment, the VD2 of the first and second polypeptide chains disclosed herein are obtained from different parent antibodies or antigen binding portions thereof.

In one embodiment the first parent antibody or antigen binding portion thereof, and the second parent antibody or antigen binding portion thereof, are the same antibody. In another embodiment the first parent antibody or antigen binding portion thereof, and the second parent antibody or antigen binding portion thereof, are different antibodies.

In one embodiment the first parent antibody or antigen binding portion thereof, binds a first antigen and the second parent antibody or antigen binding portion thereof, binds a second antigen. In a particular embodiment, the first and second antigens are the same antigen. In another embodiment, the parent antibodies bind different epitopes on the same antigen. In another embodiment the first and second antigens are different antigens. In another embodiment, the first parent antibody or antigen binding portion thereof, binds the first antigen with a potency different from the potency with which the second parent antibody or antigen binding portion thereof, binds the second antigen. In yet another embodiment, the first parent antibody or antigen binding portion thereof, binds the first antigen with an affinity different from the affinity with which the second parent antibody or antigen binding portion thereof, binds the second antigen.

In another embodiment the first parent antibody or antigen binding portion thereof, and the second parent antibody or antigen binding portion thereof, are selected from the group consisting of, human antibody, CDR grafted antibody, and humanized antibody. In an embodiment, the antigen binding portions are selected from the group consisting of a Fab fragment, a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, a dAb fragment, an isolated complementarity determining region (CDR), a single chain antibody, and diabodies.

In another embodiment the binding protein of the disclosure possesses at least one desired property exhibited by the first parent antibody or antigen binding portion thereof, or the second parent antibody or antigen binding portion thereof. Alternatively, the first parent antibody or antigen binding portion thereof and the second parent antibody or antigen binding portion thereof possess at least one desired property exhibited by the Dual Variable Domain Immunoglobulin. In an embodiment, the desired property is selected from one or more antibody parameters. In another embodiment, the antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.In an embodiment the binding protein is multivalent. In another embodiment, the binding protein is multispecific. The multivalent and or multispecific binding proteins described herein have desirable properties particularly from a therapeutic standpoint. For instance, the multivalent and or multispecific binding protein may (1) be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind; (2) be an agonist antibody; and/or (3) induce cell death and/or apoptosis of a cell expressing an antigen which the multivalent antibody is capable of binding to. The "parent antibody" which provides at least one antigen binding specificity of the multivalent and or multispecific binding proteins may be one which is internalized (and/or catabolized) by a cell expressing an antigen to which the antibody binds; and/or may be an agonist, cell death-inducing, and/or apoptosis-inducing antibody, and the multivalent and or multispecific binding protein as described herein may display improvement(s) in one or more of these properties. Moreover, the parent antibody may lack any one or more of these properties, but may be endowed with them when constructed as a multivalent binding protein as described herein.

In another embodiment the binding protein of the disclosure has an on rate constant (Kon) to one or more targets selected from the group consisting of: at least about $10^2 M^{-1}s^{-1}$; at least about $10^3 M^{-1}s^{-1}$; at least about $10^4 M^{-1}s^{-1}$; at least about $10^5 M^{-1}s^{-1}$; and at least about $10^6 M^{-1}s^{-1}$, as measured by surface plasmon resonance. In an embodiment, the binding protein of the disclosure has an on rate constant (Kon) to one or more targets between $10^2 M^{-1}s^{-1}$ and $10^3 M^{-1}s^{-1}$; between

$10^3M^{-1}s^{-1}$ and $10^4M^{-1}s^{-1}$; between $10^4M^{-1}s^{-1}$ and $10^5M^{-1}s^{-1}$; or between $10^5M^{-1}s^{-1}$ and $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance.

In another embodiment the binding protein has an off rate constant (Koff) for one or more targets selected from the group consisting of: at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; and at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance. In an embodiment, the binding protein of the disclosure has an off rate constant (Koff) to one or more targets of $10^{-3}s^{-1}$ to $10^{-4}s^{-1}$; of $10^{-4}s^{-1}$ to $10^{-5}s^{-1}$; or of $10^{-5}s^{-1}$ to $10^{-6}s^{-1}$, as measured by surface plasmon resonance.

In another embodiment the binding protein has a dissociation constant (K_D) to one or more targets selected from the group consisting of: at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; and at most 10^{-13} M. In an embodiment, the binding protein of the disclosure has a dissociation constant (K_D) to its targets of 10^{-7} M to 10^{-8} M; of 10^{-8} M to 10^{-9} M; of 10^{-9} M to 10^{-10} M; of 10^{-10} to 10^{-11} M; of 10^{-11} M to 10^{-12} M; or of 10^{-12} to M 10^{-13} M.

In another embodiment, the binding protein described herein is a conjugate further comprising an agent selected from the group consisting of an immunoadhesion molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent. In an embodiment, the imaging agent is selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin. In another embodiment, the imaging agent is a radiolabel selected from the group consisting of: ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm. In yet another embodiment, the therapeutic or cytotoxic agent is selected from the group consisting of an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.

In another embodiment, the binding protein described herein is a crystallized binding protein and exists as a crystal. In an embodiment, the crystal is a carrier-free pharmaceutical controlled release crystal. In yet another embodiment, the crystallized binding protein has a greater half life in vivo than the soluble counterpart of said binding protein. In still another embodiment, the crystallized binding protein retains biological activity.

In another embodiment, the binding protein described herein is glycosylated. For example, the glycosylation is a human glycosylation pattern.

One aspect of the disclosure pertains to an isolated nucleic acid encoding any one of the binding proteins disclosed herein. A further embodiment provides a vector comprising the isolated nucleic acid disclosed herein wherein said vector is selected from the group consisting of pcDNA; pTT (Durocher et al., *Nucleic Acids Research* 2002, Vol 30, No.2); pTT3 (pTT with

additional multiple cloning site; pEFBOS (Mizushima, S. and Nagata, S., (1990) *Nucleic acids Research* Vol 18, No. 17); pBV; pJV; pcDNA3.1 TOPO, pEF6 TOPO and pBJ. In an embodiment, the vector is a vector disclosed in US Patent Application Serial No. 61/021,282.

In another aspect a host cell is transformed with the vector disclosed herein. In an embodiment, the host cell is a prokaryotic cell. In another embodiment, the host cell is E.Coli. In a related embodiment the host cell is a eukaryotic cell. In another embodiment, the eukaryotic cell is selected from the group consisting of protist cell, animal cell, plant cell and fungal cell. In yet another embodiment, the host cell is a mammalian cell including, but not limited to, CHO, COS; NS0, SP2, PER.C6 or a fungal cell such as Saccharomyces cerevisiae; or an insect cell such as Sf9.

Another aspect of the disclosure provides a method of producing a binding protein disclosed herein comprising culturing any one of the host cells also disclosed herein in a culture medium under conditions sufficient to produce the binding protein. In an embodiment, 50%-75% of the binding protein produced by this method is a dual specific tetravalent binding protein. In a particular embodiment, 75%-90% of the binding protein produced by this method is a dual specific tetravalent binding protein. In a particular embodiment, 90%-95% of the binding protein produced is a dual specific tetravalent binding protein.

One embodiment provides a composition for the release of a binding protein wherein the composition comprises a formulation that in turn comprises a crystallized binding protein, as disclosed herein, and an ingredient, and at least one polymeric carrier. For example, the polymeric carrier is a polymer selected from one or more of the group consisting of: poly (acrylic acid), poly (cyanoacrylates), poly (amino acids), poly (anhydrides), poly (depsipeptide), poly (esters), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (b-hydroxybutryate), poly (caprolactone), poly (dioxanone); poly (ethylene glycol), poly ((hydroxypropyl) methacrylamide, poly [(organo)phosphazene], poly (ortho esters), poly (vinyl alcohol), poly (vinylpyrrolidone), maleic anhydride- alkyl vinyl ether copolymers, pluronic polyols, albumin, alginate, cellulose and cellulose derivatives, collagen, fibrin, gelatin, hyaluronic acid, oligosaccharides, glycaminoglycans, sulfated polyeaccharides, blends and copolymers thereof. For example, the ingredient is selected from the group consisting of albumin, sucrose, trehalose, lactitol, gelatin, hydroxypropyl-β- cyclodextrin, methoxypolyethylene glycol and polyethylene glycol. Another embodiment provides a method for treating a mammal comprising the step of administering to the mammal an effective amount of the composition disclosed herein.

The disclosure also provides a pharmaceutical composition comprising a binding protein, as disclosed herein and a pharmaceutically acceptable carrier. In a further embodiment the pharmaceutical composition comprises at least one additional therapeutic agent for treating a

disorder. For example, the additional agent is selected from the group consisting of: a therapeutic agent, an imaging agent, a cytotoxic agent, an angiogenesis inhibitor (including but not limited to an anti-VEGF antibody or a VEGF-trap), a kinase inhibitor (including but not limited to a KDR and a TIE-2 inhibitor), a co-stimulation molecule blocker (including but not limited to anti-B7.1, anti-B7.2, CTLA4-Ig, anti-CD20), an adhesion molecule blocker (including but not limited to an anti-LFA-1 antibody, an anti-E/L selectin antibody, a small molecule inhibitor), an anti-cytokine antibody or functional fragment thereof (including but not limited to an anti-IL-18, an anti-TNF, and an anti-IL-6/cytokine receptor antibody), methotrexate, cyclosporin, rapamycin, FK506, a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteriod, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, and a cytokine antagonist.

In another aspect, the disclosure provides a method for treating a human subject suffering from a disorder in which the target, or targets, capable of being bound by the binding protein disclosed herein is detrimental, comprising administering to the human subject a binding protein disclosed herein such that the activity of the target, or targets in the human subject is inhibited and one of more symptoms is alleviated or treatment is achieved. For example, the disorder is selected from the group comprising arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthopathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy,

spondyloarthopathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasulitis of the kidneys, lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjörgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, *alcohol-induced liver injury, choleosatatis, idiosyncratic liver disease, Drug-Induced hepatitis,* Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (*e.g.*, depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia

and lymphoma), Abetalipoprotemia, Acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-l- antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti cd3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aortic and peripheral aneuryisms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, Burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chronic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, Dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic ateriosclerotic disease, Diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's Syndrome in middle age, drug- induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, epstein-barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hematophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallerrorden-Spatz disease, hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis (A), His bundle arrythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement disorders, hypersensitity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, Asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza a, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia- reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney

transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant rejection, lymphederma, malaria, malignamt Lymphoma, malignant histiocytosis, malignant melanoma, meningitis, meningococcemia, metabolic/idiopathic diseases, migraine headache, mitochondrial multi.system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel Dejerine-Thomas Shi-Drager and Machado-Joseph), myasthenia gravis, mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodyplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic I muscular atrophies, neutropenic fever, non- hodgkins lymphoma, occlusion of the abdominal aorta and its branches, occlusive arterial disorders, okt3 therapy, orchitis/epidydimitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherlosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiotomy syndrome, preeclampsia, Progressive supranucleo Palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynoud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, Subacute sclerosing panencephalitis, Syncope, syphilis of the cardiovascular system, systemic anaphalaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, ,vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke- Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue.

In an embodiment, diseases that can be treated or diagnosed with the compositions and methods of the disclosure include, but are not limited to, primary and metastatic cancers, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder

and urothelium), female genital tract (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma), tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas), solid tumors arising from hematopoietic malignancies such as leukemias, and lymphomas (both Hodgkin's and non-Hodgkin's lymphomas).

In an embodiment, the antibodies of the disclosure or antigen-binding portions thereof, are used to treat cancer or in the prevention of metastases from the tumors described herein either when used alone or in combination with radiotherapy and/or other chemotherapeutic agents.

In another aspect the disclosure provides a method of treating a patient suffering from a disorder comprising the step of administering any one of the binding proteins disclosed herein before, concurrent, or after the administration of a second agent, as discussed herein. In a particular embodiment the second agent is selected from the group consisting of budenoside, epidermal growth factor, corticosteroids, cyclosporin, sulfasalazine, aminosalicylates, 6-mercaptopurine, azathioprine, metronidazole, lipoxygenase inhibitors, mesalamine, olsalazine, balsalazide, antioxidants, thromboxane inhibitors, IL-1 receptor antagonists, anti-IL-1β mAbs, anti-IL-6 or IL-6 receptor mAbs, growth factors, elastase inhibitors, pyridinyl-imidazole compounds, antibodies or agonists of TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-12, IL-13, IL-15, IL-16, IL-18, IL-23, EMAP-II, GM-CSF, FGF, and PDGF, antibodies of CD2, CD3, CD4, CD8, CD-19, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands, methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, ibuprofen, corticosteroids, prednisolone, phosphodiesterase inhibitors, adensosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, IRAK, NIK, IKK, p38, MAP kinase inhibitors, IL-1β converting enzyme inhibitors, TNFαconverting enzyme inhibitors, T-cell signalling inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors, soluble p55 TNF receptor, soluble p75 TNF receptor, sIL-1RI, sIL-1RII, sIL-6R, antiinflammatory cytokines, IL-4, IL-10, IL-11, IL-13 and TGFβ.

In a particular embodiment the pharmaceutical compositions disclosed herein are administered to the patient by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac,

intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

One aspect of the disclosure provides at least one anti-idiotype antibody to at least one binding protein of the present disclosure. The anti-idiotype antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule such as, but not limited to, at least one complementarily determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into a binding protein of the present disclosure.

Brief Description of the Drawings

Figure 1A is a schematic representation of Dual Variable Domain (DVD)-Ig constructs and shows the strategy for generation of a DVD-Ig from two parent antibodies;

Figure 1B, is a schematic representation of constructs DVD1-Ig, DVD2-Ig, and two chimeric mono-specific antibodies from hybridoma clones 2D13.E3 (anti-IL–1α) and 13F5.G5 (anti-IL–1β).

Detailed Description of the Invention

This invention pertains to multivalent and/or multispecific binding proteins capable of binding two or more antigens. Specifically, the invention relates to dual variable domain immunoglobulins (DVD-Ig), and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such DVD-Igs. Methods of using the DVD-Igs of the invention to detect specific antigens, either in vitro or in vivo are also encompassed by the invention.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear, however, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

That the present invention may be more readily understood, select terms are defined below.

The term "Polypeptide" as used herein, refers to any polymeric chain of amino acids. The terms "peptide" and "protein" are used interchangeably with the term polypeptide and also refer to a polymeric chain of amino acids. The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide that by virtue of its origin or source of derivation is not associated with naturally associated components that accompany it in its native state; is substantially free of other proteins from the same species; is expressed by a cell from a different species; or does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

The term "recovering" as used herein, refers to the process of rendering a chemical species such as a polypeptide substantially free of naturally associated components by isolation, e.g., using protein purification techniques well known in the art.

"Biological activity " as used herein, refers to any one or more inherent biological properties of a molecule. Biological properties include but are not limited to binding receptor; induction of cell proliferation, inhibiting cell growth, inductions of other cytokines, induction of *apoptosis, and enzymatic activity.*

The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

The term "antibody", as used herein, broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art. Nonlimiting embodiments of which are discussed below.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG 1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass.

The term "Fc region" is used to define the C-terminal region of an immunoglobulin heavy chain, which may be generated by papain digestion of an intact antibody. The Fc region may be a native sequence Fc region or a variant Fc region. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain. Replacements of amino acid residues in the Fc portion to alter antibody effector function are known in the art (Winter, *et al.* US Patent Nos 5,648,260 and 5,624,821). The Fc portion of an antibody mediates several important effector functions e.g.,cytokine induction, ADCC, phagocytosis, complement dependent cytotoxicity (CDC) and half-life/ clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. In still

another embodiment at least one amino acid residue is replaced in the constant region of the antibody, for example the Fc region of the antibody, such that effector functions of the antibody are altered. The dimerization of two identical heavy chains of an immunoglobulin is mediated by the dimerization of CH3 domains and is stabilized by the disulfide bonds within the hinge region (Huber et al. Nature; 264: 415-20; Thies et al 1999 J Mol Biol; 293: 67-79.). Mutation of cysteine residues within the hinge regions to prevent heavy chain-heavy chain disulfide bonds will destabilize dimeration of CH3 domains. Residues responsible for CH3 dimerization have been identified (Dall'Acqua 1998 Biochemistry 37: 9266-73.). Therefore, it is possible to generate a monovalent half-Ig. Interestingly, these monovalent half Ig molecules have been found in nature for both IgG and IgA subclasses (Seligman 1978 Ann Immunol 129: 855-70;Biewenga et al 1983 Clin Exp Immunol 51: 395-400). The stoichiometry of FcRn: Ig Fc region has been determined to be 2:1 (West et al .2000 Biochemistry 39: 9698-708), and half Fc is sufficient for mediating FcRn binding (Kim et al 1994 Eur J Immunol; 24: 542-548.). Mutations to disrupt the dimerization of CH3 domain may not have greater adverse effect on its FcRn binding as the residues important for CH3 dimerization are located on the inner interface of CH3 b sheet structure, whereas the region responsible for FcRn binding is located on the outside interface of CH2-CH3 domains. However the half Ig molecule may have certain advantage in tissue penetration due to its smaller size than that of a regular antibody. In one embodiment at least one amino acid residue is replaced in the constant region of the binding protein of the invention, for example the Fc region, such that the dimerization of the heavy chains is disrupted, resulting in half DVD Ig molecules.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546, Winter et al., PCT publication WO 90/05144 A1 herein incorporated by reference), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein

chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5). In addition single chain antibodies also include "linear antibodies" comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata et al. Protein Eng. 8(10):1057-1062 (1995); and US Patent No. 5,641,870).

The term "multivalent binding protein" is used throughout this specification to denote a binding protein comprising two or more antigen binding sites. In an embodiment, the multivalent binding protein is engineered to have the three or more antigen binding sites, and is generally not a naturally occurring antibody. The term "multispecific binding protein" refers to a binding protein capable of binding two or more related or unrelated targets. Dual variable domain (DVD) binding proteins of the invention comprise two or more antigen binding sites and are tetravalent or multivalent binding proteins. DVDs may be monospecific, i.e., capable of binding one antigen or multispecific, i.e. capable of binding two or more antigens. DVD binding proteins comprising two heavy chain DVD polypeptides and two light chain DVD polypeptides are referred to as DVD-Ig. Each half of a DVD-Ig comprises a heavy chain DVD polypeptide, and a light chain DVD polypeptide, and two antigen binding sites. Each binding site comprises a heavy chain variable domain and a light chain variable domain with a total of 6 CDRs involved in antigen binding per antigen binding site.

The term "bispecific antibody", as used herein, refers to full-length antibodies that are generated by quadroma technology (see Milstein, C. and A.C. Cuello, Nature, 1983. 305(5934): p. 537-40), by chemical conjugation of two different monoclonal antibodies (see Staerz, U.D., et al., Nature, 1985. 314(6012): p. 628-31), or by knob-into-hole or similar approaches which introduces mutations in the Fc region (see Holliger, P., T. Prospero, and G. Winter, Proc Natl Acad Sci U S A, 1993. 90(14): p. 6444-8.18), resulting in multiple different immunoglobulin species of which only one is the functional bispecific antibody. By molecular function, a bispecific antibody binds one antigen (or epitope) on one of its two binding arms (one pair of HC/LC), and binds a different

antigen (or epitope) on its second arm (a different pair of HC/LC). By this definition, a bispecific antibody has two distinct antigen binding arms (in both specificity and CDR sequences), and is monovalent for each antigen it binds to.

The term "dual-specific antibody", as used herein, refers to full-length antibodies that can bind two different antigens (or epitopes) in each of its two binding arms (a pair of HC/LC) (see PCT publication WO 02/02773). Accordingly a dual-specific binding protein has two identical antigen binding arms, with identical specificity and identical CDR sequences, and is bivalent for each antigen it binds to.

A "functional antigen binding site" of a binding protein is one that is capable of binding a target antigen. The antigen binding affinity of the antigen binding site is not necessarily as strong as the parent antibody from which the antigen binding site is derived, but the ability to bind antigen must be measurable using any one of a variety of methods known for evaluating antibody binding to an antigen. Moreover, the antigen binding affinity of each of the antigen binding sites of a multivalent antibody herein need not be quantitatively the same.

The term "cytokine" is a generic term for proteins released by one cell population, which act on another cell population as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-alpha and - beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-alpha; platelet-growth factor; placental growth factor, transforming growth factors (TGFs) such as TGF- alpha and TGF-beta; insulin-like growth factor-1 and -11; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, -beta and -gamma colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-18, IL-21, IL-22, IL-23, IL-33; a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

The term "linker" is used to denote polypeptides comprising two or more amino acid residues joined by peptide bonds and are used to link one or more antigen binding portions. Such

linker polypeptides are well known in the art (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Exemplary linkers include, but are not limited to, AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6); RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAAA$(G_4S)_4$ (SEQ ID NO: 9); SAKTTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15); QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP (SEQ ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22), GGGGSGGGGSGGGGS (SEQ ID NO: 23); GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25); GHEAAAVMQVQYPAS (SEQ ID NO: 26)..

An immunoglobulin constant domain refers to a heavy or light chain constant domain. Human IgG heavy chain and light chain constant domain amino acid sequences are known in the art.

The term "monoclonal antibody" or "mAb" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described

further in Section II C, below), antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom H.R. (1997) TIB Tech. 15:62-70; Azzazy H., and Highsmith W.E. (2002) Clin. Biochem. 35:425-445; Gavilondo J.V., and Larrick J.W. (2002) BioTechniques 29:128-145; Hoogenboom H., and Chames P. (2000) Immunology Today 21:371-378), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see, Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann S-A. and Green L.L. (2002) Current Opinion in Biotechnology 13:593-597; Little M. et al. (2000) Immunology Today 21:364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

An "affinity matured" antibody is an antibody with one or more alterations in one or more CDRs thereof which result an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). Exemplary affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. BidlTechnology 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas et al. Proc Nat. Acad. Sci, USA 91:3809-3813 (1994); Schier et al. Gene 169:147- 155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); Hawkins et al, J. Mol. BioL 226:889-896 (1992) and selective mutation at selective mutagenesis positions, contact or hypermutation positions with an activity enhancing amino acid residue as described in US patent US 6914128B1.

The term "chimeric antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

The term "CDR-grafted antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of another species, such as

antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs (e.g., CDR3) has been replaced with human CDR sequences.

The term "humanized antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a non-human species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", i.e., more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences. Also "humanized antibody"is an antibody or a variant, derivative, analog or fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab') 2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In an embodiment, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

The terms "Kabat numbering", "Kabat definitions" and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (*i.e.* hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat *et al.* (1971) *Ann. NY Acad. Sci.* 190:382-391 and, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable

region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia &Lesk, J. Mol. Biol. 196:901-917 (1987) and Chothia et al., Nature 342:877-883 (1989)) found that certain sub- portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (FASEB J. 9:133-139 (1995)) and MacCallum (J Mol Biol 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the herein systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although certain embodiments use Kabat or Chothia defined CDRs.

As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, -L2, and -L3 of light chain and CDR-H1, -H2, and -H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FRs represents two or more of the four sub- regions constituting a framework region.

As used herein, the term "germline antibody gene" or "gene fragment" refers to an immunoglobulin sequence encoded by non- lymphoid cells that have not undergone the maturation process that leads to genetic rearrangement and mutation for expression of a particular immunoglobulin. (See, e.g., Shapiro et al., Crit. Rev. Immunol. 22(3): 183-200 (2002); Marchalonis et al., Adv Exp Med Biol. 484:13-30 (2001)). One of the advantages provided by various embodiments of the present invention stems from the recognition that germline antibody genes are more likely than mature antibody genes to conserve essential amino acid sequence structures characteristic of individuals in the species, hence less likely to be recognized as from a foreign source when used therapeutically in that species.

As used herein, the term "neutralizing" refers to counteracting the biological activity of an antigen when a binding protein specifically binds the antigen. In an embodiment, the neutralizing binding protein binds the cytokine and reduces its biologically activity by at least about 20%, 40%, 60%, 80%, 85% or more.

The term "activity" includes activities such as the binding specificity and affinity of a DVD-Ig for two or more antigens.

The term "epitope" includes any polypeptide determinant capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, an antibody is said to specifically bind an antigen when it recognizes its target antigen in a complex mixture of proteins and/or macromolecules. Antibodies are said to "bind to the same epitope" if the antibodies cross-compete (one prevents the binding or modulating effect of the other). In addition structural definitions of epitopes (overlapping, similar, identical) are informative, but functional definitions are often more relevant as they encompass structural (binding) and functional (modulation, competition) parameters.

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jönsson, U., *et al.* (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., *et al.* (1991) *Biotechniques* 11:620-627; Johnsson, B., *et al.* (1995) *J. Mol. Recognit.* 8:125-131; and Johnnson, B., *et al.* (1991) *Anal. Biochem.* 198:268-277.

The term "K_{on}", as used herein, is intended to refer to the on rate constant for association of a binding protein (e.g., an antibody) to the antigen to form the, e.g., antibody/antigen complex as is known in the art.

The term "K_{off}", as used herein, is intended to refer to the off rate constant for dissociation of a binding protein (e.g., an antibody) from the, e.g., antibody/antigen complex as is known in the art.

The term "K_d", as used herein, is intended to refer to the dissociation constant of a particular binding protein (e.g., an antibody)-antigen interaction as is known in the art.

The term "labeled binding protein" as used herein, refers to a protein with a label incorporated that provides for the identification of the binding protein. In an embodiment, the label is a detectable marker, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm); fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags); and magnetic agents, such as gadolinium chelates.

The term "conjugate" refers to a binding protein, such as an antibody, chemically linked to a second chemical moiety, such as a therapeutic or cytotoxic agent. The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. In an embodiment, the therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

The terms "crystal" and "crystallized" as used herein, refer to a binding protein (e.g., an antibody), or antigen binding portion thereof, that exists in the form of a crystal. Crystals are one form of the solid state of matter, which is distinct from other forms such as the amorphous solid state or the liquid crystalline state. Crystals are composed of regular, repeating, three-dimensional arrays of atoms, ions, molecules (e.g., proteins such as antibodies), or molecular assemblies (e.g., antigen/antibody complexes). These three-dimensional arrays are arranged

according to specific mathematical relationships that are well-understood in the field. The fundamental unit, or building block, that is repeated in a crystal is called the asymmetric unit. Repetition of the asymmetric unit in an arrangement that conforms to a given, well-defined crystallographic symmetry provides the "unit cell" of the crystal. Repetition of the unit cell by regular translations in all three dimensions provides the crystal. See Giege, R. and Ducruix, A. Barrett, Crystallization of Nucleic Acids and Proteins, a Practical Approach, 2nd ea., pp. 20 1-16, Oxford University Press, New York, New York, (1999)."

The term "polynucleotide" means a polymeric form of two or more nucleotides, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

The term "isolated polynucleotide" shall mean a polynucleotide (e.g., of genomic, cDNA, or synthetic origin, or some combination thereof) that, by virtue of its origin, the "isolated polynucleotide" is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature; is operably linked to a polynucleotide that it is not linked to in nature; or does not occur in nature as part of a larger sequence.

The term "vector", is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. "Operably linked"

sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. The term "expression control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

"Transformation", refers to any process by which exogenous DNA enters a host cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

The term "recombinant host cell" (or simply "host cell"), is intended to refer to a cell into which exogenous DNA has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell, but, to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. In an embodiment, host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life. In another embodiment, eukaryotic cells include protist, fungal, plant and animal cells. In another embodiment, host cells include but are not limited to the prokaryotic cell line E.Coli; mammalian cell lines CHO, HEK 293, COS, NS0, SP2 and PER.C6; the insect cell line Sf9; and the fungal cell *Saccharomyces cerevisiae*.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose.

"Transgenic organism", as known in the art, refers to an organism having cells that contain a transgene, wherein the transgene introduced into the organism (or an ancestor of the organism) expresses a polypeptide not naturally expressed in the organism. A "transgene" is a DNA construct, which is stably and operably integrated into the genome of a cell from which a transgenic organism develops, directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic organism.

The term "regulate"and "modulate" are used interchangeably, and, as used herein, refers to a change or an alteration in the activity of a molecule of interest (e.g., the biological activity of a cytokine). Modulation may be an increase or a decrease in the magnitude of a certain activity or function of the molecule of interest. Exemplary activities and functions of a molecule include, but are not limited to, binding characteristics, enzymatic activity, cell receptor activation, and signal transduction.

Correspondingly, the term "modulator" is a compound capable of changing or altering an activity or function of a molecule of interest (e.g., the biological activity of a cytokine). For example, a modulator may cause an increase or decrease in the magnitude of a certain activity or function of a molecule compared to the magnitude of the activity or function observed in the absence of the modulator. In certain embodiments, a modulator is an inhibitor, which decreases the magnitude of at least one activity or function of a molecule. Exemplary inhibitors include, but are not limited to, proteins, peptides, antibodies, peptibodies, carbohydrates or small organic molecules. Peptibodies are described, e.g., in WO01/83525.

The term "agonist", refers to a modulator that, when contacted with a molecule of interest, causes an increase in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the agonist. Particular agonists of interest may include, but are not limited to, polypeptides, nucleic acids, carbohydrates, or any other molecules that bind to the antigen.

The term "antagonist" or "inhibitor", refer to a modulator that, when contacted with a molecule of interest causes a decrease in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the antagonist. Particular antagonists of interest include those that block or modulate the biological or immunological activity of of the antigen. Antagonists and inhibitors of antigens may include, but are not limited to, proteins, nucleic acids, carbohydrates, or any other molecules, which bind to the antigen.

As used herein, the term "effective amount" refers to the amount of a therapy which is sufficient to reduce or ameliorate the severity and/or duration of a disorder or one or more symptoms thereof, prevent the advancement of a disorder, cause regression of a disorder, prevent the recurrence, development, onset or progression of one or more symptoms associated with a disorder, detect a disorder, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (e.g., prophylactic or therapeutic agent).

The term "sample", as used herein, is used in its broadest sense. A "biological sample", as used herein, includes, but is not limited to, any quantity of a substance from a living thing or formerly living thing. Such living things include, but are not limited to, humans, mice, rats, monkeys, dogs, rabbits and other animals. Such substances include, but are not limited to, blood, serum, urine, synovial fluid, cells, organs, tissues, bone marrow, lymph nodes and spleen.

I. Generation of DVD binding protein

The invention pertains to Dual Variable Domain binding proteins capable of binding one or more targets and methods of making the same. In an embodiment, the binding protein comprises a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first variable domain, VD2 is a second variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1. The binding protein of the invention can be generated using various techniques. The invention provides expression vectors, host cell and methods of generating the binding protein.

A. Generation of parent monoclonal antibodies

The variable domains of the DVD binding protein can be obtained from parent antibodies, including polyclonal and mAbs capable of binding antigens of interest. These antibodies may be naturally occurring or may be generated by recombinant technology.

MAbs can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, mAbs can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al. , Antibodies: A Laboratory Manual, (Cold Spring Harbor

Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Hybridomas are selected, cloned and further screened for desirable characteristics, including robust hybridoma growth, high antibody production and desirable antibody characteristics, as discussed in Example 1below. Hybridomas may be cultured and expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro*. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art. In a particular embodiment, the hybridomas are mouse hybridomas. In another embodiment, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas, in which a human non-secretory myeloma is fused with a human cell expressing an antibody capable of binding a specific antigen.

Recombinant mAbs are also generated from single, isolated lymphocytes using a procedure referred to in the art as the selected lymphocyte antibody method (SLAM), as described in U.S. Patent No. 5,627,052, PCT Publication WO 92/02551 and Babcock, J.S. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:7843-7848. In this method, single cells secreting antibodies of interest, *e.g.*, lymphocytes derived from an immunized animal, are identified, and, heavy- and light-chain variable region cDNAs are rescued from the cells by reverse transcriptase-PCR and these variable regions can then be expressed, in the context of appropriate immunoglobulin constant regions (*e.g.*, human constant regions), in mammalian host cells, such as COS or CHO cells. The host cells transfected with the amplified immunoglobulin sequences, derived from *in vivo* selected lymphocytes, can then undergo further analysis and selection *in vitro*, for example by panning the transfected cells to isolate cells expressing antibodies to the antigen of interest. The amplified immunoglobulin sequences further can be manipulated *in vitro*, such as by *in vitro* affinity maturation methods such as those described in PCT Publication WO 97/29131 and PCT Publication WO 00/56772.

Monoclonal antibodies are also produced by immunizing a non-human animal comprising some, or all, of the human immunoglobulin locus with an antigen of interest. In an embodiment, the non-human animal is a XENOMOUSE transgenic mouse, an engineered mouse strain that comprises large fragments of the human immunoglobulin loci and is deficient in mouse antibody production. *See, e.g.,* Green et al. *Nature Genetics* 7:13-21 (1994) and United States Patents Nos. 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598 and 6,130,364. See also WO 91/10741, published July 25,1991, WO 94/02602,

published February 3, 1994, WO 96/34096 and WO 96/33735, both published October 31, 1996, WO 98/16654, published April 23, 1998, WO 98/24893, published June 11, 1998, WO 98/50433, published November 12, 1998, WO 99/45031, published September 10, 1999, WO 99/53049, published October 21, 1999, WO 00 09560, published February 24, 2000 and WO 00/037504, published June 29, 2000. The XENOMOUSE transgenic mouse produces an adult-like human repertoire of fully human antibodies, and generates antigen-specific human monoclonal antibodies. The XENOMOUSE transgenic mouse contains approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and x light chain loci. See Mendez et al., *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J. Exp. Med.* 188:483-495 (1998), the disclosures of which are hereby incorporated by reference.

In vitro methods also can be used to make the parent antibodies, wherein an antibody library is screened to identify an antibody having the desired binding specificity. Methods for such screening of recombinant antibody libraries are well known in the art and include methods described in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT Publication No. WO 92/18619; Dower *et al.* PCT Publication No. WO 91/17271; Winter *et al.* PCT Publication No. WO 92/20791; Markland *et al.* PCT Publication No. WO 92/15679; Breitling *et al.* PCT Publication No. WO 93/01288; McCafferty *et al.* PCT Publication No. WO 92/01047; Garrard *et al.* PCT Publication No. WO 92/09690; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; McCafferty *et al.*, *Nature* (1990) 348:552-554; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982, US patent application publication 20030186374, and PCT Publication No. WO 97/29131, the contents of each of which are incorporated herein by reference.

Parent antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e. g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that

can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780, 225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the herein references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies including human antibodies or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. 4,946,778 and 5,258, 498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988).

Alternative to screening of recombinant antibody libraries by phage display, other methodologies known in the art for screening large combinatorial libraries can be applied to the identification of parent antibodies. One type of alternative expression system is one in which the recombinant antibody library is expressed as RNA-protein fusions, as described in PCT Publication No. WO 98/31700 by Szostak and Roberts, and in Roberts, R.W. and Szostak, J.W. (1997) *Proc. Natl. Acad. Sci. USA* 94:12297-12302. In this system, a covalent fusion is created between an mRNA and the peptide or protein that it encodes by *in vitro* translation of synthetic mRNAs that carry puromycin, a peptidyl acceptor antibiotic, at their 3' end. Thus, a specific mRNA can be enriched from a complex mixture of mRNAs (*e.g.*, a combinatorial library) based on the properties of the encoded peptide or protein, *e.g.*, antibody, or portion thereof, such as binding of the antibody, or portion thereof, to the dual specificity antigen. Nucleic acid sequences encoding antibodies, or portions thereof, recovered from screening of such libraries can be expressed by recombinant means as described herein (*e.g.*, in mammalian host cells) and, moreover, can be subjected to further affinity maturation by either additional rounds of screening of mRNA-peptide fusions in which mutations have been introduced into the originally selected

sequence(s), or by other methods for affinity maturation *in vitro* of recombinant antibodies, as described herein.

In another approach the parent antibodies can also be generated using yeast display methods known in the art. In yeast display methods, genetic methods are used to tether antibody domains to the yeast cell wall and display them on the surface of yeast. In particular, such yeast can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Examples of yeast display methods that can be used to make the parent antibodies include those disclosed in Wittrup, et al. U.S. Patent No. 6,699,658 incorporated herein by reference.

The antibodies described herein can be further modified to generate CDR grafted and humanized parent antibodies. CDR-grafted parent antibodies comprise heavy and light chain variable region sequences from a human antibody wherein one or more of the CDR regions of V_H and/or V_L are replaced with CDR sequences of murine antibodies capable of binding antigen of interest. A framework sequence from any human antibody may serve as the template for CDR grafting. However, straight chain replacement onto such a framework often leads to some loss of binding affinity to the antigen. The more homologous a human antibody is to the original murine antibody, the less likely the possibility that combining the murine CDRs with the human framework will introduce distortions in the CDRs that could reduce affinity. Therefore, in an embodiment, the human variable framework that is chosen to replace the murine variable framework apart from the CDRs have at least a 65% sequence identity with the murine antibody variable region framework. In an embodiment, the human and murine variable regions apart from the CDRs have at least 70% sequence identify. In a particular embodiment, that the human and murine variable regions apart from the CDRs have at least 75% sequence identity. In another embodiment, the human and murine variable regions apart from the CDRs have at least 80% sequence identity. Methods for producing such antibodies are known in the art (see EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,352); and anti-idiotypic antibodies.

Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Known human Ig sequences are disclosed, e.g., www.ncbi.nlm.nih.gov/entrez- /query.fcgi; www.atcc.org/phage/hdb.html; www.sciquest.com/; www.abcam.com/; www.antibodyresource.com/onlinecomp.html; www.public.iastate.edu/.about.pedro/research_tools.html; www.mgen.uni-

heidelberg.de/SD/IT/IT.html; www.whfreeman.com/immunology/CH- 05/kuby05.htm; www.library.thinkquest.org/12429/Immune/Antibody.html; www.hhmi.org/grants/lectures/1996/vlab/; www.path.cam.ac.uk/.about.mrc7/m- ikeimages.html; www.antibodyresource.com/; mcb.harvard.edu/BioLinks/Immunology.html.www.immunologylink.com/; pathbox.wustl.edu/.about.hcenter/index.- html; www.biotech.ufl.edu/.about.hcl/; www.pebio.com/pa/340913/340913.html- ; www.nal.usda.gov/awic/pubs/antibody/; www.m.ehime-u.acjp/.about.yasuhito- /Elisa.html; www.biodesign.com/table.asp; www.icnet.uk/axp/facs/davies/lin- ks.html; www.biotech.ufl.edu/.about.fccl/protocol.html; www.isac-net.org/sites_geo.html; aximtl.imt.uni-marburg.de/.about.rek/AEP- Start.html; baserv.uci.kun.nl/.about.jraats/linksl.html; www.recab.uni-hd.de/immuno.bme.nwu.edu/; www.mrc-cpe.cam.ac.uk/imt-doc/public/INTRO.html; www.ibt.unam.mx/vir/V_mice.html; imgt.cnusc.fr:8104/; www.biochem.ucl.ac.uk/.about.martin/abs/index.html; antibody.bath.ac.uk/; abgen.cvm.tamu.edu/lab/wwwabgen.html; www.unizh.ch/.about.honegger/AHOsem-inar/Slide01.html; www.cryst.bbk.ac.uk/.about.ubcg07s/; www.nimr.mrc.ac.uk/CC/ccaewg/ccaewg.htm; www.path.cam.ac.uk/.about.mrc7/humanisation/TAHHP.html; www.ibt.unam.mx/vir/structure/stat_aim.html; www.biosci.missouri.edu/smithgp/index.html; www.cryst.bioc.cam.ac.uk/.abo- ut.fmolina/Webpages/Pept/spottech.html; www.jerini.de/fr roducts.htm; www.patents.ibm.com/ibm.html.Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983), each entirely incorporated herein by reference. Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art.

Framework residues in the human framework regions may be substituted with the corresponding residue from the CDR donor antibody to alter, e.g., improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from

the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Antibodies can be humanized using a variety of techniques known in the art, such as but not limited to those described in Jones et al., Nature 321:522 (1986); Verhoeyen et al., Science 239:1534 (1988)), Sims et al., J. Immunol. 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987), Carter et al., Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993), Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al. , PNAS 91:969-973 (1994); PCT publication WO 91/09967, PCT/: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334, GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, EP 592,106; EP 519,596, EP 239,400, U.S. Pat. Nos. 5,565,332, 5,723,323, 5,976,862, 5,824,514, 5,817,483, 5814476, 5763192, 5723323, 5,766886, 5,714,352, 6,204,023, 6,180,370, 5,693,762, 5,530,101, 5,585,089, 5,225,539; 4,816,567, each entirely incorporated herein by reference, included references cited therein.

B. Criteria for selecting parent monoclonal antibodies

An embodiment of the invention pertains to selecting parent antibodies with at least one or more properties desired in the DVD-Ig molecule. In an embodiment, the desired property is selected from one or more antibody parameters. In another embodiment, the antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.

B1. Affinity to Antigen

The desired affinity of a therapeutic mAb may depend upon the nature of the antigen, and the desired therapeutic end-point. In an embodiment, monoclonal antibodies have higher affinities (Kd = 0.01 – 0.50 pM) when blocking a cytokine-cytokine receptor interaction as such interaction are usually high affinity interactions (e.g.,<pM – <nM ranges). In such instances, the mAb affinity for its target should be equal to or better than the affinity of the cytokine (ligand) for its receptor. On the other hand, mAb with lesser affinity (> nM range) could be therapeutically effective e.g.,in clearing circulating potentially pathogenic proteins e.g.,monoclonal antibodies that bind to, sequester, and clear circulating species of A-β amyloid. In other instances, reducing the affinity of an existing high affinity mAb by site-directed mutagenesis or using a mAb with lower affinity for its target could be used to avoid potential side-effects e.g.,a high affinity mAb may sequester/neutralize all of its intended target, thereby completely depleting/eliminating the

function(s) of the targeted protein. In this scenario, a low affinity mAb may sequester/neutralize a fraction of the target that may be responsible for the disease symptoms (the pathological or over-produced levels), thus allowing a fraction of the target to continue to perform its normal physiological function(s). Therefore, it may be possible to reduce the Kd to adjust dose and/or reduce side-effects. The affinity of the parental mAb might play a role in appropriately targeting cell surface molecules to achieve desired therapeutic out-come. For example, if a target is expressed on cancer cells with high density and on normal cells with low density, a lower affinity mAb will bind a greater number of targets on tumor cells than normal cells, resulting in tumor cell elimination via ADCC or CDC, and therefore might have therapeutically desirable effects. Thus selecting a mAb with desired affinity may be relevant for both soluble and surface targets.

Signaling through a receptor upon interaction with its ligand may depend upon the affinity of the receptor-ligand interaction. Similarly, it is conceivable that the affinity of a mAb for a surface receptor could determine the nature of intracellular signaling and whether the mAb may deliver an agonist or an antagonist signal. The affinity-based nature of mAb-mediated signaling may have an impact of its side-effect profile. Therefore, the desired affinity and desired functions of therapeutic monoclonal antibodies need to be determined carefully by in vitro and in vivo experimentation.

The desired Kd of a binding protein (e.g., an antibody) may be determined experimentally depending on the desired therapeutic outcome. In an embodiment parent antibodies with affinity (Kd) for a particular antigen equal to, or better than, the desired affinity of the DVD-Ig for the same antigen are selected. The antigen binding affinity and kinetics are assessed by Biacore or another similar technique. In one embodiment, each parent antibody has a dissociation constant (Kd) to its antigen selected from the group consisting of: at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; and at most 10^{-13} M. First parent antibody from which VD1 is obtained and second parent antibody from which VD2 is obtained may have similar or different affinity (K_D) for the respective antigen. Each parent antibody has an on rate constant (Kon) to the antigen selected from the group consisting of: at least about $10^2 M^{-1}s^{-1}$; at least about $10^3 M^{-1}s^{-1}$; at least about $10^4 M^{-1}s^{-1}$; at least about $10^5 M^{-1}s^{-1}$; and at least about $10^6 M^{-1}s^{-1}$, as measured by surface plasmon resonance. The first parent antibody from which VD1 is obtained and the second parent antibody from which VD2 is obtained may have similar or different on rate constant (Kon) for the respective antigen. In one embodiment, each parent antibody has an off rate constant (Koff) to the antigen selected from the group consisting of: at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; and at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance. The first parent antibody from which VD1 is obtained and the second parent antibody from which VD2 is obtained may have similar or different off rate constants (Koff) for the respective antigen.

B2. Potency

The desired affinity/potency of parental monoclonal antibodies will depend on the desired therapeutic outcome. For example, for receptor-ligand (R-L) interactions the affinity (kd) is equal to or better than the R-L kd (pM range). For simple clearance of a pathologic circulating protein, the kd could be in low nM range e.g., clearance of various species of circulating A-β peptide. In addition, the kd will also depend on whether the target expresses multiple copies of the same epitope e.g a mAb targeting conformational epitope in Aβ oligomers.

Where VDI and VD2 bind the same antigen, but distint epitopes, the DVD-Ig will contain 4 binding sites for the same antigen, thus increasing avidity and thereby the apparent kd of the DVD-Ig. In an embodiment, parent antibodies with equal or lower kd than that desired in the DVD-Ig are chosen. The affinity considerations of a parental mAb may also depend upon whether the DVD-Ig contains four or more identical antigen binding sites (i.e; a DVD-Ig from a single mAb). In this case, the apparent kd would be greater than the mAb due to avidity. Such DVD-Igs can be employed for cross-linking surface receptor, increase neutralization potency, enhance clearance of pathological proteins etc.

In an embodiment parent antibodies with neutralization potency for specific antigen equal to or better than the desired neutralization potential of the DVD-Ig for the same antigen are selected. The neutralization potency can be assessed by a target-dependent bioassay where cells of appropriate type produce a measurable signal (i.e. proliferation or cytokine production) in response to target stimulation, and target neutralization by the mAb can reduce the signal in a dose-dependent manner.

B3. Biological functions

Monoclonal antibodies can perform potentially several functions. Some of these functions are listed in Table 1. These functions can be assessed by both in vitro assays (e.g., cell-based and biochemical assays) and in vivo animal models.

Table 1: Some Potential Applications For Therapeutic Antibodies

Target (Class)	Mechanism of Action (target)
Soluble (cytokines,other)	Neutralization of activity (e.g., a cytokine) Enhance clearance (e.g., Aβ oligomers) Increase half-life (e.g., GLP 1)
Cell Surface (Receptors, other)	Agonist (e.g., GLP1 R; EPO R; etc.) Antagonist (e.g., integrins; etc.) Cytotoxic (CD 20; etc.)
Protein deposits	Enhance clearance/degradation (e.g., Aβ plaques, amyloid deposits)

MAbs with distinct functions described in the examples herein in Table 1 can be selected to achieve desired therapeutic outcomes. Two or more selected parent monoclonal antibodies can then be used in DVD-Ig format to achieve two distinct functions in a single DVD-Ig molecule. For example, a DVD-Ig can be generated by selecting a parent mAb that neutralizes function of a specific cytokine, and selecting a parent mAb that enhances clearance of a pathological protein. Similarly, we can select two parent monoclonal antibodies that recognize two different cell surface receptors, one mAb with an agonist function on one receptor and the other mAb with an antagonist function on a different receptor. These two selected monoclonal antibodies each with a distinct function can be used to construct a single DVD-Ig molecule that will possess the two distinct functions (agonist and antagonist) of the selected monoclonal antibodies in a single molecule. Similarly, two antagonistic monoclonal antibodies to cell surface receptors each blocking binding of respective receptor ligands (e.g.,EGF and IGF) can be used in a DVD-Ig format. Conversely, an antagonistic anti-receptor mAb (e.g., anti-EGFR) and a neutralizing anti-soluble mediator (e.g., anti-IGF1/2) mAb can be selected to make a DVD-Ig.

B4. Epitope Recognition:

Different regions of proteins may perform different functions. For example specific regions of a cytokine interact with the cytokine receptor to bring about receptor activation whereas other regions of the protein may be required for stabilizing the cytokine. In this instance one may select a mAb that binds specifically to the receptor interacting region(s) on the cytokine and thereby block cytokine-receptor interaction. In some cases, for example certain chemokine receptors that bind multiple ligands, a mAb that binds to the epitope (region on chemokine receptor) that interacts with only one ligand can be selected. In other instances, monoclonal antibodies can bind to epitopes on a target that are not directly responsible for physiological functions of the protein, but binding of a mAb to these regions could either interfere with physiological functions (steric hindrance) or alter the conformation of the protein such that the protein cannot function (mAb to receptors with multiple ligand which alter the receptor conformation such that none of the ligand can bind). Anti-cytokine monoclonal antibodies that do not block binding of the cytokine to its receptor, but block signal transduction have also been identified (e.g., 125-2H, an anti-IL-18 mAb).

Examples of epitopes and mAb functions include, but are not limited to, blocking Receptor-Ligand (R-L) interaction (neutralizing mAb that binds R-interacting site); steric hindrance resulting in diminished or no R-binding. An Ab can bind the target at a site other than a receptor binding site, but still interferes with receptor binding and functions of the target by inducing conformational change and eliminate function (e.g., Xolair), binding to R but block signaling (125-2H).

In an embodiment, the parental mAb needs to target the appropriate epitope for maximum efficacy. Such epitope should be conserved in the DVD-Ig. The binding epitope of a mAb can be determined by several approaches, including co-crystallography, limited proteolysis of mAb-antigen complex plus mass spectrometric peptide mapping (Legros V. et al 2000 Protein Sci. 9:1002-10), phage displayed peptide libraries (O'Connor KH et al 2005 J Immunol Methods. 299:21-35), as well as mutagenesis (Wu C. et al . 2003 J Immunol 170:5571-7).

B5. Physicochemical and pharmaceutical properties:

Therapeutic treatment with antibodies often requires administration of high doses, often several mg/kg (due to a low potency on a mass basis as a consequence of a typically large molecular weight). In order to accommodate patient compliance and to adequately address chronic disease therapies and outpatient treatment, subcutaneous (s.c.) or intramuscular (i.m.) administration of therapeutic mAbs is desirable. For example, the maximum desirable volume for s.c. administration is ~1.0 mL, and therefore, concentrations of >100 mg/mL are desirable to limit the number of injections per dose. In an embodiment, the therapeutic antibody is administered in one dose. The development of such formulations is constrained, however, by protein-protein interactions (e.g., aggregation, which potentially increases immunogenicity risks) and by limitations during processing and delivery (e.g., viscosity). Consequently, the large quantities required for clinical efficacy and the associated development constraints limit full exploitation of the potential of antibody formulation and s.c. administration in high-dose regimens. It is apparent that the physicochemical and pharmaceutical properties of a protein molecule and the protein solution are of utmost importance, e.g.,stability, solubility and viscosity features.

B5.1. Stability:

A "stable" antibody formulation is one in which the antibody therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Stability can be measured at a selected temperature for a selected time period. In an embodiment,, the antibody in the formulation is stable at room temperature (about 30°C) or at 40°C for at least 1 month and/or stable at about 2-8°C. for at least 1 year for at least 2 years. Furthermore, in an embodiment, the formulation is stable following freezing (to, e.g., -70°C) and thawing of the formulation, hereinafter referred to as a "freeze/thaw cycle." In another example, a "stable" formulation may be one wherein less than about 10% and less than about 5% of the protein is present as an aggregate in the formulation.

A DVD-Ig stable in vitro at various temperatures for an extended time period is desirable. One can achieve this by rapid screening of parental mAbs stable in vitro at elevated temperature, e.g.,at 40°C for 2-4 weeks, and then assess stability. During storage at 2-8°C, the protein reveals stability for at least 12 months, e.g., at least 24 months. Stability (% of monomeric, intact

molecule) can be assessed using various techniques such as cation exchange chromatography, size exclusion chromatography, SDS-PAGE, as well as bioactivity testing. For a more comprehensive list of analytical techniques that may be employed to analyze covalent and conformational modifications please see Jones, A. J. S. (1993) Analytical methods for the assessment of protein formulations and delivery systems. In: Cleland, J. L.; Langer, R., editors. Formulation and delivery of peptides and proteins, 1st edition, Washington, ACS, pg. 22-45; and Pearlman, R.; Nguyen, T. H.(1990) Analysis of protein drugs. In: Lee, V. H., editor. Peptide and protein drug delivery, 1st edition, New York, Marcel Dekker, Inc., pg. 247-301.

Heterogeneity and aggregate formation: stability of the antibody may be such that the formulation may reveal less than about 10%, and, in an embodiment, less than about 5%, in another embodiment, less than about 2%, or, in an embodiment, within the range of 0.5% to 1.5% or less in the GMP antibody material that is present as aggregate. Size exclusion chromatography is a method that is sensitive, reproducible, and very robust in the detection of protein aggregates.

In addition to low aggregate levels, the antibody must , in an embodiment, be chemically stable. Chemical stability may be determined by ion exchange chromatography (e.g.,cation or anion exchange chromatography), hydrophobic interaction chromatography, or other methods such as isoelectric focusing or capillary electrophoresis. For instance, chemical stability of the antibody may be such that after storage of at least 12 months at 2-8°C the peak representing unmodified antibody in a cation exchange chromatography may increase not more than 20%, in an embodiment, not more than 10%, or, in another embodiment, not more than 5% as compared to the antibody solution prior to storage testing.

In an embodiment, the parent antibodies display structural integrity; correct disulfide bond formation, and correct folding: Chemical instability due to changes in secondary or tertiary structure of an antibody may impact antibody activity. For instance, stability as indicated by activity of the antibody may be such that after storage of at least 12 months at 2-8°C the activity of the antibody may decrease not more than 50%, in an embodiment not more than 30%, or even not more than 10%, or in an embodiment not more than 5% or 1% as compared to the antibody solution prior to storage testing. Suitable antigen-binding assays can be employed to determine antibody activity.

B5.2. Solubility:

The "solubility" of a mAb correlates with the production of correctly folded, monomeric IgG. The solubility of the IgG may therefore be assessed by HPLC. For example, soluble (monomeric) IgG will give rise to a single peak on the HPLC chromatograph, whereas insoluble (e.g., multimeric and aggregated) will give rise to a plurality of peaks. A person skilled in the art will therefore be able to detect an increase or decrease in solubility of an IgG using routine HPLC

techniques. For a more comprehensive list of analytical techniques that may be employed to analyze solubility (see Jones, A. G. Dep. Chem. Biochem. Eng., Univ. Coll. London, London, UK. Editor(s): Shamlou, P. Ayazi. Process. Solid-Liq. Suspensions (1993), 93-117. Publisher: Butterworth-Heinemann, Oxford, UK and Pearlman, Rodney; Nguyen, Tue H, Advances in Parenteral Sciences (1990), 4 (Pept. Protein Drug Delivery), 247-301). Solubility of a therapeutic mAb is critical for formulating to high concentration often required for adequate dosing. As outlined herein, solubilities of >100 mg/mL may be required to accommodate efficient antibody dosing. For instance, antibody solubility may be not less than about 5 mg/mL in early research phase, in an embodiment not less than about 25 mg/mL in advanced process science stages, or in an embodiment not less than about 100 mg/mL, or in an embodiment not less than about 150 mg/mL. It is obvious to a person skilled in the art that the intrinsic properties of a protein molecule are important the physico-chemical properties of the protein solution, e.g., stability, solubility, viscosity. However, a person skilled in the art will appreciate that a broad variety of excipients exist that may be used as additives to beneficially impact the characteristics of the final protein formulation. These excipients may include: (i) liquid solvents, cosolvents (e.g.,alcohols such as ethanol); (ii) buffering agents (e.g.,phosphate, acetate, citrate, amino acid buffers); (iii) sugars or sugar alcohols (e.g.,sucrose, trehalose, fructose, raffinose, mannitol, sorbitol, dextrans); (iv) surfactants (e.g.,polysorbate 20, 40, 60, 80, poloxamers); (v) isotonicity modifiers (e.g.,salts such as NaCl, sugars, sugar alcohols); and (vi) others (e.g.,preservatives, chelating agents, antioxidants, chelating substances (e.g.,EDTA), biodegradable polymers, carrier molecules (e.g.,HSA, PEGs)

Viscosity is a parameter of high importance with regard to antibody manufacture and antibody processing (e.g., diafiltration/ultrafiltration), fill-finish processes (pumping aspects, filtration aspects) and delivery aspects (syringeability, sophisticated device delivery). Low viscosities enable the liquid solution of the antibody having a higher concentration. This enables the same dose may be administered in smaller volumes. Small injection volumes inhere the advantage of lower pain on injection sensations, and the solutions not necessarily have to be isotonic to reduce pain on injection in the patient. The viscosity of the antibody solution may be such that at shear rates of 100 (1/s) antibody solution viscosity is below 200 mPa s, in an embodiment below 125 mPa s, in another embodiment below 70 mPa s, and in yet another embodiment below 25 mPa s or even below 10 mPa s.

B 5.3. Production efficiency

The generation of a DVD-Ig that is efficiently expressed in mammalian cells, such as Chinese hamster ovary cells (CHO), will in an embodiment require two parental monoclonal antibodies which are themselves expressed efficiently in mammalian cells. The production yield from a stable mammalian line (i.e. CHO) should be above 0.5g/L, in an embodiment above 1g/L,

and in another embodiment in the range of 2-5 g/L or more (Kipriyanov SM, Little M. 1999 Mol Biotechnol. 12:173-201; Carroll S, Al-Rubeai M. 2004 Expert Opin Biol Ther. 4:1821-9).

Production of antibodies and Ig fusion proteins in mammalian cells is influenced by several factors. Engineering of the expression vector via incorporation of strong promoters, enhancers and selection markers can maximize transcription of the gene of interest from an integrated vector copy. The identification of vector integration sites that are permissive for high levels of gene transcription can augment protein expression from a vector (Wurm et al, 2004, Nature Biotechnology, 2004, Vol/Iss/Pg. 22/11 (1393-1398)). Furthermore, levels of production are affected by the ratio of antibody heavy and light chains and various steps in the process of protein assembly and secretion (Jiang et al. 2006, Biotechnology Progress, Jan-Feb 2006, vol. 22, no. 1, p. 313-8).

B 6. Immunogenicity

Administration of a therapeutic mAb may results in certain incidence of an immune response (ie, the formation of endogenous antibodies directed against the therapeutic mAb). Potential elements that might induce immunogenicity should be analyzed during selection of the parental monoclonal antibodies, and steps to reduce such risk can be taken to optimize the parental monoclonal antibodies prior to DVD-Ig construction. Mouse-derived antibodies have been found to be highly immunogenic in patients. The generation of chimeric antibodies comprised of mouse variable and human constant regions presents a logical next step to reduce the immunogenicity of therapeutic antibodies (Morrison and Schlom, 1990). Alternatively, immunogenicity can be reduced by transferring murine CDR sequences into a human antibody framework (reshaping/CDR grafting/humanization), as described for a therapeutic antibody by Riechmann et al., 1988. Another method is referred to as "resurfacing" or "veneering", starting with the rodent variable light and heavy domains, only surface-accessible framework amino acids are altered to human ones, while the CDR and buried amino acids remain from the parental rodent antibody (Roguska et al., 1996). In another type of humanization, instead of grafting the entire CDRs, one technique grafts only the "specificity-determining regions" (SDRs), defined as the subset of CDR residues that are involved in binding of the antibody to its target (Kashmiri et al., 2005). This necessitates identification of the SDRs either through analysis of available three-dimensional structures of antibody-target complexes or mutational analysis of the antibody CDR residues to determine which interact with the target. Alternatively, fully human antibodies may have reduced immunogenicity compared to murine, chimeric or humanized antibodies.

Another approach to reduce the immunogenicity of therapeutic antibodies is the elimination of certain specific sequences that are predicted to be immunogenic. In one approach, after a first generation biologic has been tested in humans and found to be unacceptably

immunogenic, the B-cell epitopes can be mapped and then altered to avoid immune detection. Another approach uses methods to predict and remove potential T-cell epitopes. Computational methods have been developed to scan and to identify the peptide sequences of biologic therapeutics with the potential to bind to MHC proteins (Desmet et al., 2005). Alternatively a human dendritic cell-based method can be used to identify $CD4^+$ T-cell epitopes in potential protein allergens (Stickler et al., 2005; S.L. Morrison and J. Schlom, *Important Adv. Oncol.* (1990), pp. 3–18; Riechmann, L., Clark, M., Waldmann, H. and Winter, G. "*Reshaping human antibodies for therapy.*" Nature (1988) 332: 323-327; Roguska-M-A, Pedersen-J-T, Henry-A-H, Searle-S-M, Roja-C-M, Avery-B, Hoffee-M, Cook-S, Lambert-J-M, Blättler-W-A, Rees-A-R, Guild-B-C. A comparison of two murine mAbs humanized by CDR-grafting and variable domain resurfacing.Protein engineering, {Protein-Eng}, 1996, vol. 9, p. 895-904; Kashmiri-Syed-V-S, De-Pascalis-Roberto, Gonzales-Noreen-R, Schlom-Jeffrey. SDR grafting--a new approach to antibody humanization. Methods (San Diego Calif.), {Methods}, May 2005, vol. 36, no. 1, p. 25-34; Desmet-Johan, Meersseman-Geert, Boutonnet-Nathalie, Pletinckx-Jurgen, De-Clercq-Krista, Debulpaep-Maja, Braeckman-Tessa, Lasters-Ignace. Anchor profiles of HLA-specific peptides: analysis by a novel affinity scoring method and experimental validation. Proteins, 2005, vol. 58, p. 53-69; Stickler-M-M, Estell-D-A, Harding-F-A. CD4+ T-cell epitope determination using unexposed human donor peripheral blood mononuclear cells. Journal of immunotherapy 2000, vol. 23, p. 654-60.)

B 7. In vivo efficacy

To generate a DVD-Ig molecule with desired in vivo efficacy, it is important to generate and select mAbs with similarly desired in vivo efficacy when given in combination. However, in some instances the DVD-Ig may exhibit in vivo efficacy that cannot be achieved with the combination of two separate mAbs. For instance, a DVD-Ig may bring two targets in close proximity leading to an activity that cannot be achieved with the combination of two separate mAbs. Additional desirable biological functions are described herein in section **B 3**. Parent antibodies with characteristics desirable in the DVD-Ig molecule may be selected based on factors *such as pharmacokinetic t ½; tissue distribution; soluble versus cell surface targets; and target* concentration- soluble/density –surface.

B 8. In vivo tissue distribution

To generate a DVD-Ig molecule with desired in vivo tissue distribution, in an embodiment parent mAbs with similar desired in vivo tissue distribution profile must be selected. Alternatively, based on the mechanism of the dual-specific targeting strategy, it may at other times not be required to select parent mAbs with the similarly desired in vivo tissue distribution when given in combination. For instance, in the case of a DVD-Ig in which one binding

component targets the DVD-Ig to a specific site thereby bringing the second binding component to the same target site. For example, one binding specificity of a DVD-Ig could target pancreas (islet cells) and the other specificity could bring GLP1 to the pancreas to induce insulin.

B 9. Isotype:

To generate a DVD-Ig molecule with desired properties including, but not limited to, Isotype, Effector functions and the circulating half-life, in an embodiment parent mAbs with appropriate Fc-effector functions depending on the therapeutic utility and the desired therapeutic end-point are selected. There are five main heavy-chain classes or isotypes some of which have several sub-types and these determine the effector functions of an antibody molecule. These effector functions reside in the hinge region, CH2 and CH3 domains of the antibody molecule. However, residues in other parts of an antibody molecule may have effects on effector functions as well. The hinge region Fc-effector functions include: (i) antibody-dependent cellular cytotoxicity, (ii) complement (C1q) binding, activation and complement-dependent cytotoxicity (CDC), (iii) phagocytosis/clearance of antigen-antibody complexes, and (iv) cytokine release in some instances. These Fc-effector functions of an antibody molecule are mediated through the interaction of the Fc-region with a set of class-specific cell surface receptors. Antibodies of the IgG1 isotype are most active while IgG2 and IgG4 having minimal or no effector functions. The effector functions of the IgG antibodies are mediated through interactions with three structurally homologous cellular Fc receptor types (and sub-types) (FcgR1, FcgRII and FcgRIII). These effector functions of an IgG1 can be eliminated by mutating specific amino acid residues in the lower hinge region (e.g.,L234A, L235A) that are required for FcgR and C1q binding. Amino acid residues in the Fc region, in particular the CH2-CH3 domains, also determine the circulating half-life of the antibody molecule. This Fc function is mediated through the binding of the Fc-region to the neonatal Fc receptor (FcRn) which is responsible for recycling of antibody molecules from the acidic lysosomes back to the general circulation.

Whether a mAb should have an active or an inactive isotype will depend on the desired therapeutic end-point for an antibody. Some examples of usage of isotypes and desired therapeutic outcome are listed below:

a) If the desired end-point is functional neutralization of a soluble cytokine then an inactive isotype may be used;

b) If the desired out-come is clearance of a pathological protein an active isotype may be used;

c) If the desired out-come is clearance of protein aggregates an active isotype may be used;

d) If the desired outcome is to antagonize a surface receptor an inactive isotype is used (Tysabri, IgG4; OKT3, mutated IgG1);

e) If the desired outcome is to eliminate target cells an active isotype is used (Herceptin, IgG1 (and with enhanced effector functions); and

f) If the desired outcome is to clear proteins from circulation without entering the CNS an IgM isotype may be used (e.g.,clearing circulating Ab peptide species).

The Fc effector functions of a parental mAb can be determined by various in vitro methods well known in the art.

As discussed, the selection of isotype, and thereby the effector functions will depend upon the desired therapeutic end-point. In cases where simple neutralization of a circulating target is desired, for example blocking receptor-ligand interactions, the effector functions may not be required. In such instances isotypes or mutations in the Fc-region of an antibody that eliminate effector functions are desirable. In other instances where elimination of target cells is the therapeutic end-point, for example elimination of tumor cells, isotypes or mutations or de-fucosylation in the Fc-region that enhance effector functions are desirable (Presta GL, Adv. Drug Delivery Rev. 58:640-656, 2006; Satoh M., Iida S., Shitara K. Expert Opinion Biol. Ther. 6:1161-1173, 2006). Similarly, depending up on the therapeutic utility, the circulating half-life of an antibody molecule can be reduced/prolonged by modulating antibody-FcRn interactions by introducing specific mutations in the Fc region (Dall'Acqua WF, Kiener PA, Wu H. J. Biol. Chem. 281:23514-23524, 2006; Petkova SB., Akilesh S., Sproule TJ. et al. Internat. Immunol. 18:1759-1769, 2006; Vaccaro C., Bawdon R., Wanjie S et al. PNAS 103:18709-18714, 2007).

The published information on the various residues that influence the different effector functions of a normal therapeutic mAb may need to be confirmed for DVD-Ig. It may be possible that in a DVD-Ig format additional (different) Fc-region residues, other than those identified for the modulation of monoclonal antibody effector functions, may be important.

Overall, the decision as to which Fc-effector functions (isotype) will be critical in the final DVD-Ig format will depend up on the disease indication, therapeutic target, desired therapeutic end-point and safety considerations. Listed below are exemplary appropriate heavy chain and light chain constant regions including, but not limited to:

- IgG1 – allotype: G1mz
- IgG1 mutant – A234, A235
- IgG2 – allotype: G2m(n-)
- Kappa – Km3

- Lambda

Fc Receptor and C1q Studies: The possibility of unwanted antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) by antibody complexing to any overexpressed target on cell membranes can be abrogated by the (for example, L234A, L235A) hinge-region mutations. These substituted amino acids, present in the IgG1 hinge region of mAb, are expected to result in diminished binding of mAb to human Fc receptors (but not FcRn), as FcgR binding is thought to occur within overlapping sites on the IgG1 hinge region. This feature of mAb may lead to an improved safety profile over antibodies containing a wild-type IgG. Binding of mAb to human Fc receptors can be determined by flow cytometry experiments using cell lines (e.g.,THP-1, K562) and an engineered CHO cell line that expresses FcgRIIb (or other FcgRs). Compared to IgG1 control monoclonal antibodies, mAb show reduced binding to FcgRI and FcgRIIa whereas binding to FcgRIIb is unaffected. The binding and activation of C1q by antigen/IgG immune complexes triggers the classical complement cascade with consequent inflammatory and/or immunoregulatory responses. The C1q binding site on IgGs has been localized to residues within the IgG hinge region. C1q binding to increasing concentrations of mAb was assessed by C1q ELISA. The results demonstrate that mAb is unable to bind to C1q, as expected when compared to the binding of a wildtype control IgG1. Overall, the L234A, L235A hinge region mutation abolishes binding of mAb to FcgRI, FcgRIIa and C1q but does not impact the interaction of mAb with FcgRIIb. This data suggests that in vivo, mAb with mutant Fc will interact normally with the inhibitory FcgRIIb but will likely fail to interact with the activating FcgRI and FcgRIIa receptors or C1q.

Human FcRn binding: The neonatal receptor (FcRn) is responsible for transport of IgG across the placenta and to control the catabolic half-life of the IgG molecules. It might be desirable to increase the terminal half-life of an antibody to improve efficacy, to reduce the dose or frequency of administration, or to improve localization to the target. Alternatively, it might be advantageous to do the converse that is, to decrease the terminal half-life of an antibody to reduce whole body exposure or to improve the target-to-non-target binding ratios. Tailoring the interaction between IgG and its salvage receptor, FcRn, offers a way to increase or decrease the terminal half-life of IgG. Proteins in the circulation, including IgG, are taken up in the fluid phase through micropinocytosis by certain cells, such as those of the vascular endothelia. IgG can bind FcRn in endosomes under slightly acidic conditions (pH 6.0–6.5) and can recycle to the cell surface, where it is released under almost neutral conditions (pH 7.0–7.4). Mapping of the Fc-region-binding site on FcRn80, 16, 17 showed that two histidine residues that are conserved across species, His310 and His435, are responsible for the pH dependence of this interaction. Using phage-display technology, a mouse Fc-region mutation that increases binding to FcRn and extends the half-life of mouse IgG was identified (see Victor, G. et al.; Nature Biotechnology

(1997), 15(7), 637-640). Fc-region mutations that increase the binding affinity of human IgG for FcRn at pH 6.0, but not at pH 7.4, have also been identified (see Dall'Acqua William F, et al., Journal of Immunology (2002), 169(9), 5171-80). Moreover, in one case, a similar pH-dependent increase in binding (up to 27-fold) was also observed for rhesus FcRn, and this resulted in a twofold increase in serum half-life in rhesus monkeys compared with the parent IgG (see Hinton, Paul R. et al., Journal of Biological Chemistry (2004), 279(8), 6213-6216). These findings indicate that it is feasible to extend the plasma half-life of antibody therapeutics by tailoring the interaction of the Fc region with FcRn. Conversely, Fc-region mutations that attenuate interaction with FcRn can reduce antibody half-life.

B.10 Pharmacokinetics (PK):

To generate a DVD-Ig molecule with desired pharmacokinetic profile, in an embodiment parent mAbs with the similarly desired pharmacokinetic profile are selected. One consideration is that immunogenic response to monoclonal antibodies (ie, HAHA, human anti-human antibody response; HACA, human anti-chimeric antibody response) further complicates the pharmacokinetics of these therapeutic agents. In an embodiment, monoclonal antibodies with minimal or no immunogenicity are used for constructing DVD-Ig molecules such that the resulting DVD-Igs will also have minimal or no immunogenicity. Some of the factors that determine the PK of a mAb include, but are not limited to, Intrinsic properties of the mAb (VH amino acid sequence); immunogenicity; FcRn binding and Fc functions.

The PK profile of selected parental monoclonal antibodies can be easily determined in rodents as the PK profile in rodents correlates well with (or closely predicts) the PK profile of monoclonal antibodies in cynomolgus monkey and humans. The PK profile is determined as described in Example section 1.2.2.3.A.

After the parental monoclonal antibodies with desired PK characteristics (and other desired functional properties as discussed herein) are selected, the DVD-Ig is constructed. As the DVD-Ig molecules contain two antigen-binding domains from two parental monoclonal antibodies, the PK properties of the DVD-Ig are assessed as well. Therefore, while determining the PK properties of the DVD-Ig, PK assays may be employed that determine the PK profile based on functionality of both antigen-binding domains derived from the 2 parent monoclonal antibodies. The PK profile of a DVD-Ig can be determined as described in Example 1.2.2.3.A. Additional factors that may impact the PK profile of DVD-Ig include the antigen-binding domain (CDR) orientation; Linker size; and Fc / FcRn interactions. PK characteristics of parent antibodies can be evaluated by assessing the following parameters: absorption, distribution, metabolism and excretion.

Absorption: To date, administration of therapeutic monoclonal antibodies is via parenteral routes (e.g., intravenous [IV], subcutaneous [SC], or intramuscular [IM]). Absorption of a mAb into the systemic circulation following either SC or IM administration from the interstitial space is primarily through the lymphatic pathway. Saturable, presystemic, proteolytic degradation may result in variable absolute bioavailability following extravascular administration. Usually, increases in absolute bioavailability with increasing doses of monoclonal antibodies may be observed due to saturated proteolytic capacity at higher doses. The absorption process for a mAb is usually quite slow as the lymph fluid drains slowly into the vascular system, and the duration of absorption may occur over hours to several days.The absolute bioavailability of monoclonal antibodies following SC administration generally ranges from 50% to 100%.

Distribution: *Following IV administration, monoclonal antibodies usually follow a* biphasic serum (or plasma) concentration-time profile, beginning with a rapid distribution phase, followed by a slow elimination phase. In general, a biexponential pharmacokinetic model best describes this kind of pharmacokinetic profile. The volume of distribution in the central compartment (Vc) for a mAb is usually equal to or slightly larger than the plasma volume (2-3 liters). A distinct biphasic pattern in serum (plasma) concentration versus time profile may not be apparent with other parenteral routes of administration, such as IM or SC, because the distribution phase of the serum (plasma) concentration-time curve is masked by the long absorption portion. Many factors, including physicochemical properties, site-specific and target-oriented receptor mediated uptake, binding capacity of tissue, and mAb dose can influence biodistribution of a mAb. Some of these factors can contribute to nonlinearity in biodistribution for a mAb.

Metabolism and Excretion: Due to the molecular size, intact monoclonal antibodies are not excreted into the urine via kidney. They are primarily inactivated by metabolism (e.g., catabolism). For IgG-based therapeutic monoclonal antibodies, half-lives typically ranges from hours or 1-2 days to over 20 days. The elimination of a mAb can be affected by many factors, including, but not limited to, affinity for the FcRn receptor, immunogenicity of the mAb, the *degree of glycosylation of the mAb, the susceptibility for the mAb to proteolysis, and receptor-*mediated elimination.

B.11 Tissue cross-reactivity pattern on human and tox species:

Identical staining pattern suggests that potential human toxicity can be evaluated in tox species. Tox species are those animal in which unrelated toxicity is studied.

The individual antibodies are selected to meet two criteria. (1) Tissue staining appropriate for the known expression of the antibody target. (2) Similar staining pattern between human and tox species tissues from the same organ.

Criterion 1: Immunizations and/or antibody selections typically employ recombinant or synthesized antigens (proteins, carbohydrates or other molecules). Binding to the natural counterpart and counterscreen against unrelated antigens are often part of the screening funnel for therapeutic antibodies. However, screening against a multitude of antigens is often unpractical. Therefore tissue cross-reactivity studies with human tissues from all major organs serve to rule out unwanted binding of the antibody to any unrelated antigens.

Criterion 2: Comparative tissue cross reactivity studies with human and tox species tissues (cynomolgus monkey, dog, possibly rodents and others, the same 36 or 37 tissues are being tested as in the human study) help to validate the selection of a tox species. In the typical tissue cross-reactivity studies on frozen tissues sections therapeutic antibodies may demonstrate the expected binding to the known antigen and/or to a lesser degree binding to tissues based either on low level interactions (unspecific binding, low level binding to similar antigens, low level charge based interactions etc.). In any case the most relevant toxicology animal species is the one with the highest degree of coincidence of binding to human and animal tissue.

Tissue cross reactivity studies follow the appropriate regulatory guidelines including EC *CPMP Guideline III/5271/94 "Production and quality control of mAbs" and the* 1997 US FDA/CBER "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use". . Cryosections (5 μm) of human tissues obtained at autopsy or biopsy were fixed and dried on object glass. The peroxidase staining of tissue sections was performed, using the avidin-biotin system. FDA's Guidance *"Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use"*. Relevant references include Clarke J 2004, Boon L. 2002a, Boon L 2002b, Ryan A 1999.

Tissue cross reactivity studies are often done in two stages, with the first stage including cryosections of 32 tissues (typically: Adrenal Gland, Gastrointestinal Tract, Prostate, Bladder, Heart, Skeletal Muscle, Blood Cells, Kidney, Skin, Bone Marrow, Liver, Spinal Cord, Breast, Lung, Spleen, Cerebellum, Lymph Node, Testes, Cerebral Cortex, Ovary, Thymus, Colon, Pancreas, Thyroid, Endothelium, Parathyroid, Ureter, Eye, Pituitary, Uterus, Fallopian Tube and Placenta) from one human donor. In the second phase a full cross reactivity study is performed with up to 38 tissues (including adrenal, blood, blood vessel, bone marrow, cerebellum, cerebrum, cervix, esophagus, eye, heart, kidney, large intestine, liver, lung, lymph node, breast mammary gland, ovary, oviduct, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, small intestine, spinal cord, spleen, stomach, striated muscle, testis, thymus, thyroid, tonsil, ureter, urinary bladder, and uterus) from 3 unrelated adults. Studies are done typically at minimally two dose levels.

The therapeutic antibody (i.e. test article) and isotype matched control antibody may be biotinylated for avidin-biotin complex (ABC) detection; other detection methods may include tertiary antibody detection for a FITC (or otherwise) labeled test article, or precomplexing with a labeled anti-human IgG for an unlabeled test article.

Briefly, cryosections (about 5 μm) of human tissues obtained at autopsy or biopsy are fixed and dried on object glass. The peroxidase staining of tissue sections is performed, using the avidin-biotin system. First (in case of a precomplexing detection system), the test article is incubated with the secondary biotinylated anti-human IgG and developed into immune complex. The immune complex at the final concentrations of 2 and 10 μg/mL of test article is added onto tissue sections on object glass and then the tissue sections were reacted for 30 minutes with a avidin-biotin-peroxidase kit. Subsequently, DAB (3,3'-diaminobenzidine), a substrate for the peroxidase reaction, was applied for 4 minutes for tissue staining. Antigen-Sepharose beads are used as positive control tissue sections.

Any specific staining is judged to be either an expected (e.g., consistent with antigen expression) or unexpected reactivity based upon known expression of the target antigen in question. Any staining judged specific is scored for intensity and frequency. Antigen or serum competion or blocking studies can assist further in determining whether observed staining is specific or nonspecific.

If two selected antibodies are found to meet the selction criteria – appropriate tissue staining, matching staining between human and toxicology animal specific tissue – they can be selected for DVD-Ig generation.

The tissue cross reactivity study has to be repeated with the final DVD-Ig construct, but while these studies follow the same protocol as outline herein, they are more complex to evaluate because any binding can come from any of the two parent antibodies, and any unexplained binding needs to be confirmed with complex antigen competition studies.

It is readily apparent that the complex undertaking of tissue crossreactivity studies with a multispecific molecule like a DVD-Ig is greatly simplified if the two parental antibodies are selected for (1) lack of unexpected tissue cross reactivity findings and (2) for appropriate similarity of tissue cross reactivity findings between the corresponding human and toxicology animal species tissues.

B.12 Specificity and selectivity:

To generate a DVD-Ig molecule with desired specificity and selectivity, one needs to generate and select parent mAbs with the similarly desired specificity and selectivity profile.

Binding studies for specificity and selectivity with a DVD-Ig can be complex due to the four or more binding sites, two each for each antigen. Briefly, binding studies using ELISA, BIAcore. KinExA or other interaction studies with a DVD-Ig need to monitor the binding of one, two or more antigens to the DVD-Ig molecule. While BIAcore technology can resolve the sequential, independent binding of multiple antigens, more traditional methods including ELISA or more modern techniques like KinExA cannot. Therefore careful characterization of each parent antibody is critical. After each individual antibody has been characterized for specificity, confirmation of specificity retention of the individual binding sites in the DVD-Ig molecule is greatly simplified.

It is readily apparent that the complex undertaking of determining the specificity of a DVD-Ig is greatly simplified if the two parental antibodies are selected for specificity prior to being combined into a DVD-Ig.

Antigen–antibody interaction studies can take many forms, including many classical protein protein interaction studies, including ELISA (Enzyme linked immunosorbent assay), Mass spectrometry, chemical cross linking, SEC with light scattering, equilibrium dialysis, gel permeation, ultrafiltration, gel chromatography, large-zone analytical SEC, micropreparative ultracentrigugation (sedimentation equilibrium), spectroscopic methods, titration microcalorimetry, sedimentation equilibrium (in analytical ultracentrifuge), sedimentation velocity (in analytical centrifuge), surface plasmon resonance (including BIAcore). Relevant references include "Current Protocols in Protein Science", John E. Coligan, Ben M. Dunn, David W. Speicher, Paul T, Wingfield (eds.) Volume 3, chapters 19 and 20, published by John Wiley & Sons Inc., and references included therein and "Current Protocols in Immunology", John E. Coligan, Barbara E. Bierer, David H. Margulies, Ethan M. Shevach, Warren Strober (eds.) published by John Wiley & Sons Inc and relevant references included therein.

Cytokine Release in Whole Blood: The interaction of mAb with human blood cells can be investigated by a cytokine release assay (Wing, M. G. Therapeutic Immunology (1995), 2(4), 183-190; "Current Protocols in Pharmacology", S.J. Enna, Michael Williams, John W. Ferkany, Terry Kenakin, Paul Moser, (eds.) published by John Wiley & Sons Inc; Madhusudan, S. Clinical Cancer Research (2004), 10(19), 6528-6534; Cox, J. Methods (2006), 38(4), 274-282; Choi, I. European Journal of Immunology (2001), 31(1), 94-106). Briefly, various concentrations of mAb are incubated with human whole blood for 24 hours. The concentration tested should cover a wide range including final concentrations mimicking typical blood levels in patients (including but not limited to 100 ng/ml – 100μg/ml). Following the incubation, supernatants and cell lysates were analyzed for the presence of IL-1Rα, TNF-α, IL-1b, IL-6 and IL-8. Cytokine concentration profiles generated for mAb were compared to profiles produced by a negative human IgG control and a positive LPS or PHA control. The cytokine profile displayed by mAb from both cell

supernatants and cell lysates was comparable to control human IgG. In an embodiment, the monoclonal antibody does not interact with human blood cells to spontaneously release inflammatory cytokines.

Cytokine release studies for a DVD-Ig are complex due to the four or more binding sites, two each for each antigen. Briefly, cytokine release studies as described herein measure the effect of the whole DVD-Ig molecule on whole blood or other cell systems, but can resolve which portion of the molecule causes cytokine release. Once cytokine release has been detected, the purity of the DVD-Ig preparation has to be ascertained, because some co-purifying cellular components can cause cytokine release on their own. If purity is not the issue, fragmentation of DVD-Ig (including but not limited to removal of Fc portion, separation of binding sites etc.), binding site mutagenesis or other methods may need to be employed to deconvolute any observations. It is readily apparent that this complex undertaking is greatly simplified if the two parental antibodies are selected for lack of cytokine release prior to being combined into a DVD-Ig.

B.13 Cross reactivity to other species for toxicological studies:

In an embodiment, the individual antibodies selected with sufficient cross-reactivity to appropriate tox species, for example, cynomolgus monkey. Parental antibodies need to bind to orthologous species target (i.e. cynomolgus monkey) and elicit appropriate response (modulation, neutralization, activation). In an embodiment, the cross-reactivity (affinity/potency) to orthologous species target should be within 10-fold of the human target. In practice, the parental antibodies are evaluated for multiple species, including mouse, rat, dog, monkey (and other non-human primates), as well as disease model species (i.e. sheep for asthma model). The acceptable cross-reactivity to tox species from the perantal monoclonal antibodies allows future toxicology studies of DVD-Ig-Ig in the same species. For that reason, the two parental monoclonal antibodies should have acceptable cross-reactivity for a common tox species therefore allowing toxicology studies of DVD-Ig in the same species.

Parent mAbs may be selected from various mAbs capable of binding specific targets and well known in the art. These include, but are not limited to anti-TNF antibody (US Patent No. 6,258,562), anti-IL-12 and/or anti-IL-12p40 antibody (US Patent No. 6,914,128); anti-IL-18 antibody (US 2005/0147610 A1), anti-C5, anti-CBL, anti-CD147, anti-gp120, anti-VLA-4, anti-CD11a, anti-CD18, anti-VEGF, anti-CD40L, anti CD-40 (e.g., see WO2007124299) anti-Id, anti-ICAM-1, anti-CXCL13, anti-CD2, anti-EGFR, anti-TGF-beta 2, anti-HGF, anti-cMet, anti DLL-4, anti-NPR1, anti-PLGF, anti-ErbB3, anti-E-selectin, anti-Fact VII, anti-Her2/neu, anti-F gp, anti-CD11/18, anti-CD14, anti-ICAM-3, anti-RON, anti CD-19, anti-CD80 (e.g., see WO2003039486, anti-CD4, anti-CD3, anti-CD23, anti-beta2-integrin, anti-alpha4beta7, anti-

CD52, anti-HLA DR, anti-CD22 (e.g., see US Patent NO: 5,789,554), anti-CD20, anti-MIF, anti-CD64 (FcR), anti-TCR alpha beta, anti-CD2, anti-Hep B, anti-CA 125, anti-EpCAM, anti-gp120, anti-CMV, anti-gpIIbIIIa, anti-IgE, anti-CD25, anti-CD33, anti-HLA, anti-IGF1,2, anti-IGFR, anti-IGF1R, anti-RGMa, anti-tetanus toxoid, anti-VNRintegrin, anti-IL-1alpha, anti-IL-1beta, anti-IL-1 receptor, anti-IL-2 receptor, anti-IL-4, anti-IL-4 receptor, anti-IL5, anti-IL-5 receptor, anti-IL-6, anti-IL-8, anti-IL-9, anti-IL-13, anti-IL-13 receptor, anti-IL-17, and anti-IL-23 (see Presta LG. 2005 Selection, design, and engineering of therapeutic antibodies J Allergy Clin Immunol. 116:731-6 and http://www.path.cam.ac.uk/~mrc7/humanisation/antibodies.html).

Parent mAbs may also be selected from various therapeutic antibodies approved for use, in clinical trials, or in development for clinical use. Such therapeutic antibodies include, but are not limited to, rituximab (Rituxan®, IDEC/Genentech/Roche) (see for example U. S. Pat. No. 5,736,137), a chimeric anti-CD20 antibody approved to treat Non-Hodgkin's lymphoma; HuMax-CD20, an anti-CD20 currently being developed by Genmab, an anti-CD20 antibody described in U.S. Pat. No. 5, 500,362, AME-133 (Applied Molecular Evolution), hA20 (Immunomedics, Inc.), HumaLYM (Intracel), and PRO70769 (PCT/US2003/040426, entitled "Immunoglobulin Variants and Uses Thereof"), trastuzumab (Herceptin®, Genentech) (see for example U.S. Pat. No. 5,677,171), a humanized anti- Her2/neu antibody approved to treat breast cancer; pertuzumab (rhuMab-2C4, Omnitarg®), currently being developed by Genentech; an anti-Her2 antibody described in U.S. Pat. No. 4,753,894; cetuximab (Erbitux®, Imclone) (U.S. Pat. No. 4,943,533; PCT WO 96/40210), a chimeric anti-EGFR antibody in clinical trials for a variety of cancers; ABX-EGF (U.S. Pat. No. 6,235,883), currently being developed by Abgenix-Immunex-Amgen; HuMax- EGFr (U.S. Ser. No. 10/172,317), currently being developed by Genmab; 425, EMD55900, EMD62000, and EMD72000 (Merck KGaA) (U.S. Pat. No. 5,558,864; Murthy et al. 1987, Arch Biochem Biophys. 252(2):549-60; Rodeck et al., 1987, J Cell Biochem. 35(4):315-20; Kettleborough et al., 1991, Protein Eng. 4(7):773-83); ICR62 (Institute of Cancer Research) (PCT WO 95/20045; Modjtahedi et al., 1993, J. Cell Biophys. 1993, 22(1-3):129-46; Modjtahedi et al., 1993, Br J Cancer. 1993, 67(2):247-53; Modjtahedi et al, 1996, Br J Cancer, 73(2):228-35; Modjtahedi et al, 2003, Int J Cancer, 105(2):273-80); TheraCIM hR3 (YM Biosciences, Canada and Centro de Immunologia Molecular, Cuba (U.S. Pat. No. 5,891,996; U.S. Pat. No. 6,506, 883; Mateo et al, 1997, Immunotechnology, 3(1):71-81); mAb-806 (Ludwig Institue for Cancer Research, Memorial Sloan-Kettering) (Jungbluth et al. 2003, Proc Natl Acad Sci USA. 100(2):639-44); KSB-102 (KS Biomedix); MR1-1 (IVAX, National Cancer Institute) (PCT WO 0162931A2); and SC100 (Scancell) (PCT WO 01/88138); alemtuzumab (Campath®, Millenium), a humanized mAb currently approved for treatment of B-cell chronic lymphocytic leukemia; muromonab-CD3 (Orthoclone OKT3®), an anti-CD3 antibody developed by Ortho Biotech/Johnson & Johnson, ibritumomab tiuxetan (Zevalin®), an

anti-CD20 antibody developed by IDEC/Schering AG, gemtuzumab ozogamicin (Mylotarg®), an anti-CD33 (p67 protein) antibody developed by Celltech/Wyeth, alefacept (Amevive®), an anti-LFA-3 Fc fusion developed by Biogen), abciximab (ReoPro®), developed by Centocor/Lilly, basiliximab (Simulect®), developed by Novartis, palivizumab (Synagis®), developed by Medimmune, infliximab (Remicade®), an anti-TNFalpha antibody developed by Centocor, adalimumab (Humira®), an anti-TNFalpha antibody developed by Abbott, Humicade®, an anti-TNFalpha antibody developed by Celltech, golimumab (CNTO-148), a fully human TNF antibody developed by Centocor, etanercept (Enbrel®), an p75 TNF receptor Fc fusion developed by Immunex/Amgen, lenercept, an p55TNF receptor Fc fusion previously developed by Roche, ABX-CBL, an anti-CD147 antibody being developed by Abgenix, ABX-IL8, an anti-IL8 antibody being developed by Abgenix, ABX-MA1, an anti-MUC18 antibody being developed by Abgenix, Pemtumomab (R1549, 90Y-muHMFG1), an anti-MUC1 in development by Antisoma, Therex (R1550), an anti-MUC1 antibody being developed by Antisoma, AngioMab (AS1405), being developed by Antisoma, HuBC-1, being developed by Antisoma, Thioplatin (AS1407) being developed by Antisoma, Antegren® (natalizumab), an anti-alpha-4-beta-1 (VLA-4) and alpha-4-beta-7 antibody being developed by Biogen, VLA-1 mAb, an anti-VLA-1 integrin antibody being developed by Biogen, LTBR mAb, an anti-lymphotoxin beta receptor (LTBR) antibody being developed by Biogen, CAT-152, an anti-TGF-β2 antibody being developed by Cambridge Antibody Technology, ABT 874 (J695), an anti- IL-12 p40 antibody being developed by Abbott, CAT-192, an anti-TGFβ1 antibody being developed by Cambridge Antibody Technology and Genzyme, CAT-213, an anti-Eotaxin1 antibody being developed by Cambridge Antibody Technology, LymphoStat-B® an anti-Blys antibody being developed by Cambridge Antibody Technology and Human Genome Sciences Inc., TRAIL-R1mAb, an anti-TRAIL-R1 antibody being developed by Cambridge Antibody Technology and Human Genome Sciences, Inc. , Avastin® bevacizumab, rhuMAb-VEGF), an anti-VEGF antibody being developed by Genentech, an anti-HER receptor family antibody being developed by Genentech, Anti-Tissue Factor (ATF), an anti-Tissue Factor antibody being developed by Genentech, Xolair® (Omalizumab), an anti-IgE antibody being developed by Genentech, Raptiva® (Efalizumab), an anti- CD11a antibody being developed by Genentech and Xoma, MLN-02 Antibody (formerly LDP-02), being developed by Genentech and Millenium Pharmaceuticals, HuMax CD4, an anti-CD4 antibody being developed by Genmab, HuMax-IL15, an anti-IL15 antibody being developed by Genmab and Amgen, HuMax-Inflam, being developed by Genmab and Medarex, HuMax-Cancer, an anti-Heparanase I antibody being developed by Genmab and Medarex and Oxford GcoSciences, HuMax-Lymphoma, being developed by Genmab and Amgen, HuMax-TAC, being developed by Genmab, IDEC-131, and anti-CD40L antibody being developed by IDEC Pharmaceuticals, IDEC-151 (Clenoliximab), an anti- CD4 antibody being developed by IDEC Pharmaceuticals, IDEC-114, an anti- CD80

antibody being developed by IDEC Pharmaceuticals, IDEC-152, an anti- CD23 being developed by IDEC Pharmaceuticals, anti-macrophage migration factor (MIF) antibodies being developed by IDEC Pharmaceuticals, BEC2, an anti-idiotypic antibody being developed by Imclone, IMC-1C11, an anti-KDR antibody being developed by Imclone, DC101, an anti-flk-1 antibody being developed by Imclone, anti-VE cadherin antibodies being developed by Imclone, CEA-Cide® (labetuzumab), an anti-carcinoembryonic antigen (CEA) antibody being developed by Immunomedics, LymphoCide® (Epratuzumab), an anti-CD22 antibody being developed by Immunomedics, AFP-Cide, being developed by Immunomedics, MyelomaCide, being developed by Immunomedics, LkoCide, being developed by Immunomedics, ProstaCide, being developed by Immunomedics, MDX-010, an anti-CTLA4 antibody being developed by Medarex, MDX-060, an anti-CD30 antibody being developed by Medarex, MDX-070 being developed by Medarex, MDX-018 being developed by Medarex, Osidem® (IDM-1), and anti-Her2 antibody being developed by Medarex and Immuno-Designed Molecules, HuMax®-CD4, an anti-CD4 antibody being developed by Medarex and Genmab, HuMax-IL15, an anti-IL15 antibody being developed by Medarex and Genmab, CNTO 148, an anti-TNFα antibody being developed by Medarex and Centocor/J&J, CNTO 1275, an anti-cytokine antibody being developed by Centocor/J&J, MOR101 and MOR102, anti-intercellular adhesion molecule-1 (ICAM-1) (CD54) antibodies being developed by MorphoSys, MOR201, an anti-fibroblast growth factor receptor 3 (FGFR-3) antibody being developed by MorphoSys, Nuvion® (visilizumab), an anti-CD3 antibody being developed by Protein Design Labs, HuZAF®, an anti-gamma interferon antibody being developed by Protein Design Labs, Anti-α 5β1 Integrin, being developed by Protein Design Labs, anti-IL-12, being developed by Protein Design Labs, ING-1, an anti-Ep-CAM antibody being developed by Xoma, Xolair® (Omalizumab) a humanized anti-IgE antibody developed by Genentech and Novartis, and MLN01, an anti-Beta2 integrin antibody being developed by Xoma, all of the herein-cited references in this paragraph are expressly incorporated herein by reference. In another embodiment, the therapeutics include KRN330 (Kirin); huA33 antibody (A33, Ludwig Institute for Cancer Research); CNTO 95 (alpha V integrins, Centocor); MEDI-522 (alpha Vβ3 integrin, Medimmune); volociximab (alpha Vβ1 integrin, Biogen/PDL); Human mAb 216 (B cell glycosolated epitope, NCI); BiTE MT103 (bispecific CD19 x CD3, Medimmune); 4G7xH22 (Bispecific BcellxFcgammaR1, Medarex/Merck KGa); rM28 (Bispecific CD28 x MAPG, US Patent No. EP1444268); MDX447 (EMD 82633) (Bispecific CD64 x EGFR, Medarex); Catumaxomab (removab) (Bispecific EpCAM x anti-CD3, Trion/Fres); Ertumaxomab (bispecific HER2/CD3, Fresenius Biotech); oregovomab (OvaRex) (CA-125, ViRexx); Rencarex® (WX G250) (carbonic anhydrase IX, Wilex); CNTO 888 (CCL2, Centocor); TRC105 (CD105 (endoglin), Tracon); BMS-663513 (CD137 agonist, Brystol Myers Squibb); MDX-1342 (CD19, Medarex); Siplizumab (MEDI-507) (CD2, Medimmune); Ofatumumab (Humax-CD20) (CD20, Genmab); Rituximab (Rituxan)

(CD20, Genentech); veltuzumab (hA20) (CD20, Immunomedics); Epratuzumab (CD22, Amgen); lumiliximab (IDEC 152) (CD23, Biogen); muromonab-CD3 (CD3, Ortho); HuM291 (CD3 fc receptor, PDL Biopharma); HeFi-1, CD30, NCI); MDX-060 (CD30, Medarex); MDX-1401 (CD30, Medarex); SGN-30 (CD30, Seattle Genentics); SGN-33 (Lintuzumab) (CD33, Seattle Genentics); Zanolimumab (HuMax-CD4) (CD4, Genmab); HCD122 (CD40, Novartis); SGN-40 (CD40, Seattle Genentics); Campath1h (Alemtuzumab) (CD52, Genzyme); MDX-1411 (CD70, Medarex); hLL1 (EPB-1) (CD74.38, Immunomedics); Galiximab (IDEC-144) (CD80, Biogen); MT293 (TRC093/D93) (cleaved collagen, Tracon); HuLuc63 (CS1, PDL Pharma); ipilimumab (MDX-010) (CTLA4, Brystol Myers Squibb); Tremelimumab (Ticilimumab, CP-675,2) (CTLA4, Pfizer); HGS-ETR1 (Mapatumumab) (DR4 TRAIL-R1 agonist, Human Genome Science /Glaxo Smith Kline); AMG-655 (DR5, Amgen); Apomab (DR5, Genentech); CS-1008 (DR5, Daiichi Sankyo); HGS-ETR2 (lexatumumab) (DR5 TRAIL-R2 agonist, HGS); Cetuximab (Erbitux) (EGFR, Imclone); IMC-11F8, (EGFR, Imclone); Nimotuzumab (EGFR, YM Bio); Panitumumab (Vectabix) (EGFR, Amgen); Zalutumumab (HuMaxEGFr) (EGFR, Genmab); CDX-110 (EGFRvIII, AVANT Immunotherapeutics); adecatumumab (MT201) (Epcam , Merck); edrecolomab (Panorex, 17-1A) (Epcam , Glaxo/Centocor); MORAb-003 (folate receptor a, Morphotech); KW-2871 (ganglioside GD3, Kyowa); MORAb-009 (GP-9, Morphotech); CDX-1307 (MDX-1307) (hCGb, Celldex); Trastuzumab (Herceptin) (HER2, Celldex); Pertuzumab (rhuMAb 2C4) (HER2 (DI), Genentech); apolizumab (HLA-DR beta chain, PDL Pharma); AMG-479 (IGF-1R, Amgen); anti-IGF-1R R1507 (IGF1-R, Roche); CP 751871 (IGF1-R, Pfizer); IMC-A12 (IGF1-R, Imclone); BIIB022 (IGF-1R , Biogen); Mik-beta-1 (IL-2Rb (CD122), Hoffman LaRoche); CNTO 328 (IL6, Centocor); Anti-KIR (1-7F9) (Killer cell Ig-like Receptor (KIR), Novo); Hu3S193 (Lewis (y), Wyeth, Ludwig Institute of Cancer Research); hCBE-11 (LTβR, Biogen); HuHMFG1 (MUC1, Antisoma/NCI); RAV12 (N-linked carbohydrate epitope, Raven); CAL (parathyroid hormone-related protein (PTH-rP), University of California); CT-011 (PD1, CureTech); MDX-1106 (ono-4538) (PD1, Medarex/Ono); MAb CT-011 (PD1, Curetech); IMC-3G3 (PDGFRa, Imclone); bavituximab (phosphatidylserine, Peregrine); huJ591 (PSMA, Cornell Research Foundation); muJ591 (PSMA, Cornell Research Foundation); GC1008 (TGFb (pan) inhibitor (IgG4), Genzyme); Infliximab (Remicade) (TNFa, Centocor); A27.15 (transferrin receptor, Salk Institute, INSERN WO 2005/111082); E2.3 (transferrin receptor, Salk Institute); Bevacizumab (Avastin) (VEGF, Genentech); HuMV833 (VEGF, Tsukuba Research Lab-WO/2000/034337, University of Texas); IMC-18F1 (VEGFR1, Imclone); IMC-1121 (VEGFR2, Imclone).

B. Construction of DVD molecules:

The dual variable domain immunoglobulin (DVD-Ig) molecule is designed such that two different light chain variable domains (VL) from the two different parent monoclonal antibodies

are linked in tandem directly or via a short linker by recombinant DNA techniques, followed by the light chain constant domain. Similarly, the heavy chain comprises two different heavy chain variable domains (VH) linked in tandem, followed by the constant domain CH1 and Fc region (**Fig.1A**).

The variable domains can be obtained using recombinant DNA techniques from a parent antibody generated by any one of the methods described herein. In an embodiment, the variable domain is a murine heavy or light chain variable domain. In another embodiment, the variable domain is a CDR grafted or a humanized variable heavy or light chain domain. In an embodiment, the variable domain is a human heavy or light chain variable domain.

In one embodiment the first and second variable domains are linked directly to each other using recombinant DNA techniques. In another embodiment the variable domains are linked via a linker sequence. In an embodiment, two variable domains are linked. Three or more variable domains may also be linked directly or via a linker sequence. The variable domains may bind the same antigen or may bind different antigens. DVD molecules of the invention may include one immunoglobulin variable domain and one non- immunoglobulin variable domain such as ligand binding domain of a receptor, active domain of an enzyme. DVD molecules may also comprise 2 or more non-Ig domains.

The linker sequence may be a single amino acid or a polypeptide sequence. In an embodiment, the linker sequences are selected from the group consisting of AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6); RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAAA$(G_4S)_4$ (SEQ ID NO: 9); SAKTTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15); QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP (SEQ ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22), GGGGSGGGGSGGGGS (SEQ ID NO: 23); GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25); GHEAAAVMQVQYPAS (SEQ ID NO: 26). The choice of linker sequences is based on crystal structure analysis of several Fab molecules. There is a natural flexible linkage between the variable domain and the CH1/CL constant domain in Fab or antibody molecular structure. This natural linkage comprises approximately 10-12 amino acid residues, contributed by 4-6 residues from C-terminus of V domain and 4-6 residues from the N-terminus of CL/CH1 domain. DVD Igs of the invention were generated using N-terminal 5-6 amino acid residues, or 11-12 amino acid residues, of CL or CH1 as linker in light chain and heavy chain of DVD-Ig, respectively.

The N-terminal residues of CL or CH1 domains, particularly the first 5-6 amino acid residues, adopt a loop conformation without strong secondary structures, therefore can act as flexible linkers between the two variable domains. The N-terminal residues of CL or CH1 domains are natural extension of the variable domains, as they are part of the Ig sequences, therefore minimize to a large extent any immunogenicity potentially arising from the linkers and junctions.

Other linker sequences may include any sequence of any length of CL/CH1 domain but not all residues of CL/CH1 domain; for example the first 5-12 amino acid residues of the CL/CH1 domains; the light chain linkers can be from Cκ or Cλ; and the heavy chain linkers can be derived from CH1 of any isotypes, including Cγ1, Cγ2, Cγ3, Cγ4, Cα1, Cα2, Cδ, Cε, and Cμ. Linker sequences may also be derived from other proteins such as Ig-like proteins, (e.g.TCR, FcR, KIR); G/S based sequences (e.g G4S repeats); hinge region-derived sequences; and other natural sequences from other proteins.

In an embodiment a constant domain is linked to the two linked variable domains using recombinant DNA techniques. In an embodiment, sequence comprising linked heavy chain variable domains is linked to a heavy chain constant domain and sequence comprising linked light chain variable domains is linked to a light chain constant domain. In an embodiment, the constant domains are human heavy chain constant domain and human light chain constant domain respectively. In an embodiment, the DVD heavy chain is further linked to an Fc region. The Fc region may be a native sequence Fc region, or a variant Fc region. In another embodiment, the Fc region is a human Fc region. In another embodiment the Fc region includes Fc region from IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, or IgD.

In another embodiment two heavy chain DVD polypeptides and two light chain DVD polypeptides are combined to form a DVD-Ig molecule. Table 2 lists amino acid sequences of VH and VL regions of exemplary antibodies for targets useful for treating disease, e.g., for treating cancer. In an embodiment, the invention provides a DVD comprising at least two of the VH and/or VL regions listed in Table 2, in any orientation.

Table 2: List of Amino Acid Sequences of VH and VL regions of Antibodies for Generating DVD-Igs

SEQ ID No.	ABT Unique ID	Protein region	Sequence 12345678901234567890123456789012345678900
27	AB002VH	VH CD3	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQR PGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAY MQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS

SEQ ID No.	ABT Unique ID	Protein region	Sequence 12345678901234567890123456789012345678901234567890
28	AB002VL	VL CD3	QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSG TSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAE DAATYYCQQWSSNPLTFGSGTKLEINR
29	*AB005VH*	*VH RON*	*EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYAMHWVRQA* PGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYCARFSGWPNNYYYYGMDVWGQGTTV TVSS
30	AB005VL	VL RON	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGFNYVDW YLQKPGQSPHLLIYFGSYRASGVPDRFSGSGSGTDFTLKI SRVEAEDVGVYYCMQALQTPPWTFGQGTKVEIRR
31	AB011VH	VH IGF1R	EVQLLESGGGLVQPGGSLRLSCTASGFTFSSYAMNWVRQA PGKGLEWVSAISGSGGTTFYADSVKGRFTISRDNSRTTLY LQMNSLRAEDTAVYYCAKDLGWSDSYYYYYGMDVWGQGTT VTVSS
32	AB011VL	VL IGF1R	DIQMTQFPSSLSASVGDRVTITCRASQGIRNDLGWYQQKP GKAPKRLIYAASRLHRGVPSRFSGSGSGTEFTLTISSLQP EDFATYYCLQHNSYPCSFGQGTKLEIKR
33	AB012VH	VH HGF	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQA PGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLY LQMNSLRAEDTAVYYCARDEYNSGWYVLFDYWGQGTLVTV SS
34	AB012VL	VL HGF	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKP GKAPNLLIYEASSLQSGVPSRFGGSGSGTDFTLTISSLQP EDFATYYCQQANGFPWTFGQGTKVEIKR
35	AB014VH	VH VEGF (seq. 1)	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQA PGKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAY LQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVT VSS
36	AB014VL	VL VEGF (seq. 1)	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKP GKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQYSTVPWTFGQGTKVEIKR

SEQ ID No.	ABT Unique ID	Protein region	Sequence 12345678901234567890123456789012345678901234567890
37	AB015VH	VH DLL-4	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDNWISWVRQA PGKGLEWVGYISPNSGFTYYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCARDNFGGYFDYWGQGTLVTVSS
38	AB015VL	VL DLL-4	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKP GKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQP EDFATTYYCQQSYTGTVTFGQGTKVEIKR
39	AB033VH	VH EGFR (seq. 1)	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQS PGKGLEWLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFF KMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSA
40	AB033VL	VL EGFR (seq. 1)	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRT NGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVES EDIADYYCQQNNNWPTTFGAGTKLELKR
41	AB047VL	VH PLGF	QVQLQQSGAELVKPGASVKISCKASGYTFTDYYINWVKLA PGQGLEWIGWIYPGSGNTKYNEKFKGKATLTIDTSSSTAY MQLSSLTSEDTAVYFCVRDSPFPDYWGQGTLLTVSS
42	AB047VH	VL PLGF	DIVLTQSPDSLAVSLGERVTMNCKSSQSLLNSGMRKSFLA WYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLT ISSVQAEDVAVYYCKQSYHLFTFGSGTKLEIKR
43	AB059VH	VH-RGMa	EVQLVESGGGLVQPGSSLKLSCVASGFTFSNYGMNWIRQA PKKGLEWIGMIYYDSSEKHYADSVKGRFTISRDNSKNTLY LEMNSLRSEDTAIYYCAKGTTPDYWGQGVMVTVSS
44	AB059VL	VL-RGMa	DVVLTQTPVSLSVTLGDQASMSCRSSQSLEYSDGYTFLEW FLQKPGQSPQLLIYEVSNRFSGVPDRFIGSGSGTDFTLKI SRVEPEDLGVYYCFQATHDPLTFGSGTKLEIKR
45	AB062VH	VH ErbB3 (seq. 1)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQP PGKGLEWIGEINHSGSTNYNPSLKSRVTISVETSKNQFSL KLSSVTAADTAVYYCARDKWTWYFDLWGRGTLVTVSS
46	AB062VL	VL ErbB3 (seq. 1)	DIEMTQSPDSLAVSLGERATINCRSSQSVLYSSSNRNYLA WYQQNPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLT ISSLQAEDVAVYYCQQYYSTPRTFGQGTKVEIKR

SEQ ID No.	ABT Unique ID	Protein region	Sequence 12345678901234567890123456789012345678901234567890
47	AB063VH	VH ErbB3 (seq. 2)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSIYSMNWVRQA PGKGLEWVSYISSSSSTIYYADSVKGRFTISRDNAKNSLY LQMNSLRDEDTAVYYCARDRGDFDAFDIWGQGTMVTVSS
48	AB063VL	VL ErbB3 (seq. 2)	DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNWYQQKP GKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQP EDIATYNCQQCENFPITFGQGTRLEIKR
49	AB064VH	VH EGFR (seq. 2)	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQ PPGKGLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQFF LKLNSVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
50	AB064VL	VL EGFR (seq. 2)	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGWLQQKP GKSFKGLIYHGTNLDDGVPSRFSGSGSGTDYTLTISSLQP EDFATYYCVQYAQFPWTFGGGTKLEIKR
51	AB067VH	VH ErbB3 (seq. 3)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYVMAWVRQA PGKGLEWVSSISSSGGWTLYADSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYCTRGLKMATIFDYWGQGTLVTVSS
52	AB067VL	VL ErbB3 (seq. 3)	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNVVSWYQQ HPGKAPKLIIYEVSQRPSGVSNRFSGSKSGNTASLTISGL QTEDEADYYCCSYAGSSIFVIFGGGTKVTVLG
53	AB070VH	VH VEGF (seq. 2)	EVQLVESGGGLVQPGGSLRLSCAASGFTISDYWIHWVRQA PGKGLEWVAGITPAGGYTYYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCARFVFFLPYAMDYWGQGTLVTVSS
54	AB070VL	VL VEGF (seq. 2)	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKP GKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQSYTTPPTFGQGTKVEIKR
55	AB071VH	VH VEGF (seq. 3)	EVQLVESGGGLVQPGGSLRLSCAASGFTINASWIHWVRQA PGKGLEWVGAIYPYSGYTNYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCARWGHSTSPWAMDYWGQGTLVTVS S
56	AB071VL	VL VEGF (seq. 3)	DIQMTQSPSSLSASVGDRVTITCRASQVIRRSLAWYQQKP GKAPKLLIYAASNLASGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQSNTSPLTFGQGTKVEIKR

SEQ ID No.	ABT Unique ID	Protein region	Sequence 12345678901234567890123456789012345678901234567890
57	AB059VH	VH-RGMa	EVQLVESGGGLVQPGSSLKLSCVASGFTFSNYGMNWIRQA PKKGLEWIGMIYYDSSEKHYADSVKGRFTISRDNSKNTLY LEMNSLRSEDTAIYYCAKGTTPDYWGQGVMVTVSS
58	AB059VL	VL-RGMa	DVVLTQTPVSLSVTLGDQASMSCRSSQSLEYSDGYTFLEW FLQKPGQSPQLLIYEVSNRFSGVPDRFIGSGSGTDFTLKI SRVEPEDLGVYYCFQATHDPLTFGSGTKLEIKR
323	AB122VH	VH EGFR (seq. 3)	QVQLQESGPGLVKPSQTLSLTCTVSGYSITSDYAWNWIRQ PPGKGLEWMGYISYSGNTRYNPSLKSRITISRDTSKNQFF LKLNSVTAADTATYYCATAGRGFPYWGQGTLVTVSS
324	AB122VL	VL EGFR (seq. 3)	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGWLQQKP GKSFKGLIYHGTNLDDGVPSRFSGSGSGTDYTLTISSLQP EDFATYYCVQYGQFPWTFGGGTKLEIKR
325	AB123VH	VH EGFR (seq. 4)	QVQLQESGPGLVKPSQTLSLTCTVSGYSITSDYAWNWIRQ PPGKGLEWMGYISYSANTRYNPSLKSRITISRDTSKNQFF LKLNSVTAADTATYYCATAGRGFPYWGQGTLVTVSS
326	AB123VL	VL EGFR (seq. 4)	DIQMTQSPSSMSVSVGDRVTITCHSSQDISSNIGWLQQKP GKSFKGLIYHGTNLEDGVPSRFSGSGSGTDYTLTISSLQP EDFATYYCVQYGQFPWTFGGGTKLEIKR
327	AB124VH	VH EGFR (seq. 5)	QVQLQESGPGLVKPSQTLSLTCTVSGYSITSDYAWNWIRQ PPGKGLEWMGYISYSGNTRYNPSLRSRITISRDTSKNQFF LKLNSVTAADTATYYCATAGRGFPYWGQGTLVTVSS
328	AB124VL	VL EGFR (seq. 5)	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGWLQQKP GKSFKGLIYHGTNLDDGVPSRFSGSGSGTDYTLTISSLQP EDFATYYCVQYGQFPWTFGGGTKLEIKR

Detailed description of specific DVD-Ig molecules capable of binding specific targets, and methods of making the same, is provided in the Examples section below.

C. Production of DVD proteins

Binding proteins of the present invention may be produced by any of a number of techniques known in the art. For example, expression from host cells, wherein expression vector(s) encoding the DVD heavy and DVD light chains is (are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a

prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the DVD proteins of the invention in either prokaryotic or eukaryotic host cells, DVD proteins are expressed in eukaryotic cells, for example, mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active DVD protein.

Exemplary mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R.J. Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells, SP2 and PER.C6 cells. When recombinant expression vectors encoding DVD proteins are introduced into mammalian host cells, the DVD proteins are produced by culturing the host cells for a period of time sufficient to allow for expression of the DVD proteins in the host cells or secretion of the DVD proteins into the culture medium in which the host cells are grown. DVD proteins can be recovered from the culture medium using standard protein purification methods.

In an exemplary system for recombinant expression of DVD proteins of the invention, a recombinant expression vector encoding both the DVD heavy chain and the DVD light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the DVD heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the DVD heavy and light chains and intact DVD protein is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the DVD protein from the culture medium. Still further the invention provides a method of synthesizing a DVD protein of the invention by culturing a host cell of the invention in a suitable culture medium until a DVD protein of the invention is synthesized. The method can further comprise isolating the DVD protein from the culture medium.

An important feature of DVD-Ig is that it can be produced and purified in a similar way as a conventional antibody. The production of DVD-Ig results in a homogeneous, single major product with desired dual-specific activity, without any sequence modification of the constant region or chemical modifications of any kind. Other previously described methods to generate "bi-specific", "multi-specific", and "multi-specific multivalent" full length binding proteins do

not lead to a single primary product but instead lead to the intracellular or secreted production of a mixture of assembled inactive, mono-specific, multi-specific, multivalent, full length binding proteins, and multivalent full length binding proteins with combination of different binding sites. As an example, based on the design described by Miller and Presta (PCT publication WO2001/077342(A1), there are 16 possible combinations of heavy and light chains. Consequently only 6.25% of protein is likely to be in the desired active form, and not as a single major product or single primary product compared to the other 15 possible combinations. Separation of the desired, fully active forms of the protein from inactive and partially active forms of the protein using standard chromatography techniques, typically used in large scale manufacturing, is yet to be demonstrated.

Surprisingly the design of the "dual-specific multivalent full length binding proteins" of the present invention leads to a dual variable domain light chain and a dual variable domain heavy chain which assemble primarily to the desired "dual-specific multivalent full length binding proteins".

At least 50%, at least 75% and at least 90% of the assembled, and expressed dual variable domain immunoglobulin molecules are the desired dual-specific tetravalent protein. This aspect of the invention particularly enhances the commercial utility of the invention. Therefore, the present invention includes a method to express a dual variable domain light chain and a dual variable domain heavy chain in a single cell leading to a single primary product of a "dual-specific tetravalent full length binding protein".

The present invention provides a methods of expressing a dual variable domain light chain and a dual variable domain heavy chain in a single cell leading to a "primary product" of a "dual-specific tetravalent full length binding protein", where the "primary product" is more than 50% of all assembled protein, comprising a dual variable domain light chain and a dual variable domain heavy chain.

The present invention provides methods of expressing a dual variable domain light chain and a dual variable domain heavy chain in a single cell leading to a single "primary product" of a "dual-specific tetravalent full length binding protein", where the "primary product" is more than 75% of all assembled protein, comprising a dual variable domain light chain and a dual variable domain heavy chain.

The present invention provides methods of expressing a dual variable domain light chain and a dual variable domain heavy chain in a single cell leading to a single "primary product" of a "dual-specific tetravalent full length binding protein", where the "primary product" is more than 90% of all assembled protein, comprising a dual variable domain light chain and a dual variable domain heavy chain.

II. Derivatized DVD binding proteins:

One embodiment provides a labeled binding protein wherein the binding protein of the invention is derivatized or linked to another functional molecule (e.g., another peptide or protein). For example, a labeled binding protein of the invention can be derived by functionally linking an binding protein of the invention (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the binding protein with another molecule (such as a streptavidin core region or a polyhistidine tag).

Useful detectable agents with which a binding protein of the invention may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin and the like. A binding protein may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When a binding protein is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. a binding protein may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

Another embodiment of the invention provides a crystallized binding protein and formulations and compositions comprising such crystals. In one embodiment the crystallized binding protein has a greater half-life in vivo than the soluble counterpart of the binding protein. In another embodiment the binding protein retains biological activity after crystallization.

Crystallized binding protein of the invention may be produced according to methods known in the art and as disclosed in WO 02072636, incorporated herein by reference.

Another embodiment of the invention provides a glycosylated binding protein wherein the antibody or antigen-binding portion thereof comprises one or more carbohydrate residues. Nascent *in vivo* protein production may undergo further processing, known as post-translational modification. In particular, sugar (glycosyl) residues may be added enzymatically, a process known as glycosylation. The resulting proteins bearing covalently linked oligosaccharide side chains are known as glycosylated proteins or glycoproteins. Antibodies are glycoproteins with one or more carbohydrate residues in the Fc domain, as well as the variable domain. Carbohydrate residues in the Fc domain have important effect on the effector function of the Fc domain, with minimal effect on antigen binding or half-life of the antibody (R. Jefferis,

Biotechnol. Prog. **21** (2005), pp. 11–16). In contrast, glycosylation of the variable domain may have an effect on the antigen binding activity of the antibody. Glycosylation in the variable domain may have a negative effect on antibody binding affinity, likely due to steric hindrance (Co, M.S., et al., Mol. Immunol. (1993) 30:1361- 1367), or result in increased affinity for the antigen (Wallick, S.C., et al., Exp. Med. (1988) 168:1099-1109; Wright, A., et al., EMBO J. (1991) 10:2717 2723).

One aspect of the present invention is directed to generating glycosylation site mutants in which the O- or N-linked glycosylation site of the binding protein has been mutated. One skilled in the art can generate such mutants using standard well-known technologies. Glycosylation site mutants that retain the biological activity but have increased or decreased binding activity are another object of the present invention.

In still another embodiment, the glycosylation of the antibody or antigen-binding portion of the invention is modified. For example, an aglycoslated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in PCT Publication WO2003016466A2, and U.S. Pat. Nos. 5,714,350 and 6,350,861, each of which is incorporated herein by reference in its entirety.

Additionally or alternatively, a modified binding protein of the invention can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues (see Kanda, Yutaka et al., Journal of Biotechnology (2007), 130(3), 300-310.) or an antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. See, for example, Shields, R. L. et al. (2002) J. Biol. Chem. 277:26733-26740; Umana et al. (1999) Nat. Biotech. 17:176-1, as well as, European Patent No: EP 1,176,195; PCT Publications WO 03/035835; WO 99/54342 80, each of which is incorporated herein by reference in its entirety.

Protein glycosylation depends on the amino acid sequence of the protein of interest, as well as the host cell in which the protein is expressed. Different organisms may produce different

glycosylation enzymes (eg., glycosyltransferases and glycosidases), and have different substrates (nucleotide sugars) available. Due to such factors, protein glycosylation pattern, and composition of glycosyl residues, may differ depending on the host system in which the particular protein is expressed. Glycosyl residues useful in the invention may include, but are not limited to, glucose, galactose, mannose, fucose, n-acetylglucosamine and sialic acid. In an embodiment, the glycosylated binding protein comprises glycosyl residues such that the glycosylation pattern is human.

It is known to those skilled in the art that differing protein glycosylation may result in differing protein characteristics. For instance, the efficacy of a therapeutic protein produced in a microorganism host, such as yeast, and glycosylated utilizing the yeast endogenous pathway may be reduced compared to that of the same protein expressed in a mammalian cell, such as a CHO cell line. Such glycoproteins may also be immunogenic in humans and show reduced half-life *in vivo* after administration. Specific receptors in humans and other animals may recognize specific glycosyl residues and promote the rapid clearance of the protein from the bloodstream. Other adverse effects may include changes in protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion, recognition by other proteins or factors, antigenicity, or allergenicity. Accordingly, a practitioner may choose a therapeutic protein with a specific composition and pattern of glycosylation, for example glycosylation composition and pattern identical, or at least similar, to that produced in human cells or in the species-specific cells of the intended subject animal.

Expressing glycosylated proteins different from that of a host cell may be achieved by genetically modifying the host cell to express heterologous glycosylation enzymes. Using techniques known in the art a practitioner may generate antibodies or antigen-binding portions thereof exhibiting human protein glycosylation. For example, yeast strains have been genetically modified to express non-naturally occurring glycosylation enzymes such that glycosylated proteins (glycoproteins) produced in these yeast strains exhibit protein glycosylation identical to that of animal cells, especially human cells (U.S patent applications 20040018590 and 20020137134 and PCT publication WO2005100584 A2).

In addition to the binding proteins, the present invention is also directed to anti-idiotypic (anti-Id) antibodies specific for such binding proteins of the invention. An anti-Id antibody is an antibody, which recognizes unique determinants generally associated with the antigen-binding region of another antibody. The anti-Id can be prepared by immunizing an animal with the binding protein or a CDR containing region thereof. The immunized animal will recognize, and respond to the idiotypic determinants of the immunizing antibody and produce an anti-Id antibody. It is readily apparent that it may be easier to generate anti-idiotypic antibodies to the two or more parent antibodies incorporated into a DVD-Ig molecule; and confirm binding studies

by methods well recognized in the art (e.g.,BIAcore, ELISA) to verify that anti-idiotypic antibodies specific for the idiotype of each parent antibody also recognize the idiotype (e.g.,antigen binding site) in the context of the DVD-Ig. The anti-idiotypic antibodies specific for each of the two or more antigen binding sites of a DVD-Ig provide ideal reagents to measure DVD-Ig concentrations of a human DVD-Ig in patrient serum; DVD-Ig concentration assays can be established using a "sandwich assay ELISA format" with an antibody to a first antigen binding regions coated on the solid phase (e.g.,BIAcore chip, ELISA plate etc.), rinsed with rinsing buffer, incubation with the serum sample, another rinsing step and ultimately incubation with another anti-idiotypic antibody to the another antigen binding site, itself labeled with an enzyme for quantitation of the binding reaction. In an embodiment, for a DVD-Ig with more than two different binding sites, anti-idiotypic antibodies to the two outermost binding sites (most distal and proximal from the constant region) will not only help in determining the DVD-Ig concentration in human serum but also document the integrity of the molecule *in vivo*. Each anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody.

Further, it will be appreciated by one skilled in the art that a protein of interest may be expressed using a library of host cells genetically engineered to express various glycosylation enzymes, such that member host cells of the library produce the protein of interest with variant glycosylation patterns. A practitioner may then select and isolate the protein of interest with particular novel glycosylation patterns. In an embodiment, the protein having a particularly selected novel glycosylation pattern exhibits improved or altered biological properties.

III. Uses of DVD-Ig

Given their ability to bind to two or more antigens the binding proteins of the invention can be used to detect the antigens (*e.g.*, in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. The DVD-Ig is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm.

In an embodiment, the binding proteins of the invention are capable of neutralizing the activity of the antigens both *in vitro* and *in vivo*. Accordingly, such DVD-Igs can be used to inhibit antigen activity, *e.g.*, in a cell culture containing the antigens, in human subjects or in other mammalian subjects having the antigens with which a binding protein of the invention cross-reacts. In another embodiment, the invention provides a method for reducing antigen activity in a subject suffering from a disease or disorder in which the antigen activity is detrimental. A binding protein of the invention can be administered to a human subject for therapeutic purposes.

As used herein, the term "a disorder in which antigen activity is detrimental" is intended to include diseases and other disorders in which the presence of the antigen in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which antigen activity is detrimental is a disorder in which reduction of antigen activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of the antigen in a biological fluid of a subject suffering from the disorder (*e.g.*, an increase in the concentration of antigen in serum, plasma, synovial fluid, *etc.* of the subject). Non-limiting examples of disorders that can be treated with the binding proteins of the invention include those disorders discussed below and in the section pertaining to pharmaceutical compositions of the antibodies of the invention.

The DVD-Igs of the invention may bind one antigen or multiple antigens. Such antigens include, but are not limited to, the targets listed in the following databases, which databases are incorporated herein by reference. These target databases include those listings:

Therapeutic targets (http://xin.cz3.nus.edu.sg/group/cjttd/ttd.asp);

Cytokines and cytokine receptors (http://www.cytokinewebfacts.com/, http://www.copewithcytokines.de/cope.cgi, and

http://cmbi.bjmu.edu.cn/cmbidata/cgf/CGF_Database/cytokine.medic.kumamoto-u.ac.jp/CFC/indexR.html);

Chemokines (http://cytokine.medic.kumamoto-u.ac.jp/CFC/CK/Chemokine.html);

Chemokine receptors and GPCRs (http://csp.medic.kumamoto-u.ac.jp/CSP/Receptor.html, http://www.gpcr.org/7tm/);

Olfactory Receptors (http://senselab.med.yale.edu/senselab/ORDB/default.asp);

Receptors (http://www.iuphar-db.org/iuphar-rd/list/index.htm);

Cancer targets (http://cged.hgc.jp/cgi-bin/input.cgi);

Secreted proteins as potential antibody targets (http://spd.cbi.pku.edu.cn/);

Protein kinases (http://spd.cbi.pku.edu.cn/), and

Human CD markers (http://content.labvelocity.com/tools/6/1226/CD_table_final_locked.pdf) and (Zola H, 2005 CD molecules 2005: human cell differentiation molecules Blood, 106:3123-6).

DVD-Igs are useful as therapeutic agents to simultaneously block two different targets to enhance efficacy/safety and/or increase patient coverage. Such targets may include soluble targets (TNF) and cell surface receptor targets (VEGFR and EGFR). It can also be used to induce redirected cytotoxicity between tumor cells and T cells (Her2 and CD3) for cancer therapy, or between autoreactive cell and effector cells for autoimmune disease or transplantation, or between any target cell and effector cell to eliminate disease-causing cells in any given disease.

In addition, DVD-Ig can be used to trigger receptor clustering and activation when it is designed to target two different epitopes on the same receptor. This may have benefit in making agonistic and antagonistic anti-GPCR therapeutics. In this case, DVD-Ig can be used to target two different epitopes (including epitopes on both the loop regions and the extracellular domain) on one cell for clustering/signaling (two cell surface molecules) or signaling (on one molecule). Similarly, a DVD-Ig molecule can be designed to triger CTLA-4 ligation, and a negative signal by targeting two different epitopes (or 2 copies of the same epitope) of CTLA-4 extracellular domain, leading to down regulation of the immune response. CTLA-4 is a clinically validated target for therapeutic treatment of a number of immunological disorders. CTLA-4/B7 interactions negatively regulate T cell activation by attenuating cell cycle progression, IL-2 production, and proliferation of T cells following activation, and CTLA-4 (CD152) engagement can down-regulate T cell activation and promote the induction of immune tolerance. However, the strategy of attenuating T cell activation by agonistic antibody engagement of CTLA-4 has been unsuccessful since CTLA-4 activation requires ligation. The molecular interaction of CTLA-4/B7 is in "skewed zipper" arrays, as demonstrated by crystal structural analysis (Stamper 2001 Nature 410:608). However none of the currently available CTLA-4 binding reagents have ligation properties, including anti-CTLA-4 mAbs. There have been several attempts to address this issue. In one case, a cell member-bound single chain antibody was generated, and significantly inhibited allogeneic rejection in mice (Hwang 2002 JI 169:633). In a separate case, artificial APC surface-linked single-chain antibody to CTLA-4 was generated and demonstrated to attenuate T cell responses (Griffin 2000 JI 164:4433). In both cases, CTLA-4 ligation was achieved by closely localized member-bound antibodies in artificial systems. While these experiments provide proof-of-concept for immune down-regulation by triggering CTLA-4 negative signaling, the reagents used in these reports are not suitable for therapeutic use. To this end, CTLA-4 ligation may be achieved by using a DVD-Ig molecule, which target two different epitopes (or 2 copies of the

same epitope) of CTLA-4 extracellular domain. The rationale is that the distance spanning two binding sites of an IgG, approximately 150-170Å, is too large for active ligation of CTLA-4 (30-50 Å between 2 CTLA-4 homodimer). However the distance between the two binding sites on DVD-Ig (one arm) is much shorter, also in the range of 30-50 Å, allowing proper ligation of CTLA-4.

Similarly, DVD-Ig can target two different members of a cell surface receptor complex (e.g.,IL-12R alpha and beta). Furthermore, DVD-Ig can target CR1 and a soluble protein/pathogen to drive rapid clearance of the target soluble protein/pathogen.

Additionally, DVD-Igs of the invention can be employed for tissue-specific delivery (target a tissue marker and a disease mediator for enhanced local PK thus higher efficacy and/or lower toxicity), including intracellular delivery (targeting an internalizing receptor and a intracellular molecule), delivering to inside brain (targeting transferrin receptor and a CNS disease mediator for crossing the blood-brain barrier). DVD-Ig can also serve as a carrier protein to deliver an antigen to a specific location via binding to a non-neutralizing epitope of that antigen and also to increase the half-life of the antigen. Furthermore, DVD-Ig can be designed to either be physically linked to medical devices implanted into patients or target these medical devices (see Burke, Sandra E.; Kuntz, Richard E.; Schwartz, Lewis B., Zotarolimus eluting stents. Advanced Drug Delivery Reviews (2006), 58(3), 437-446; Surface coatings for biological activation and functionalization of medical devices, Hildebrand, H. F.; Blanchemain, N.; Mayer, G.; Chai, F.; Lefebvre, M.; Boschin, F., Surface and Coatings Technology (2006), 200(22-23), 6318-6324; Drug/ device combinations for local drug therapies and infection prophylaxis, Wu, Peng; Grainger, David W., Biomaterials (2006), 27(11), 2450-2467; Mediation of the cytokine network in the implantation of orthopedic devices., Marques, A. P.; Hunt, J. A.; Reis, Rui L., Biodegradable Systems in Tissue Engineering and Regenerative Medicine (2005), 377-397). Briefly, directing appropriate types of cell to the site of medical implant may promote healing and restoring normal tissue function. Alternatively, inhibition of mediators (including but not limited to cytokines), released upon device implantation by a DVD coupled to or target to a device is also provided. For example, Stents have been used for years in interventional cardiology to clear blocked arteries and to improve the flow of blood to the heart muscle. However, traditional bare metal stents have been known to cause restenosis (re-narrowing of the artery in a treated area) in some patients and can lead to blood clots. Recently, an anti-CD34 antibody coated stent has been described which reduced restenosis and prevents blood clots from occurring by capturing endothelial progenitor cells (EPC) circulating throughout the blood. Endothelial cells are cells that line blood vessels, allowing blood to flow smoothly. The EPCs adhere to the hard surface of the stent forming a smooth layer that not only promotes healing but prevents restenosis and blood clots, complications previously associated with the use of stents (Aoji et al. 2005 J Am Coll

Cardiol. 45(10):1574-9). In addition to improving outcomes for patients requiring stents, there are also implications for patients requiring cardiovascular bypass surgery. For example, a prosthetic vascular conduit (artificial artery) coated with anti-EPC antibodies would eliminate the need to use arteries from patients legs or arms for bypass surgery grafts. This would reduce surgery and anesthesia times, which in turn will reduce coronary surgery deaths. DVD-Ig are designed in such a way that it binds to a cell surface marker (such as CD34) as well as a protein (or an epitope of any kind, including but not limited to proteins, lipids and polysaccharides) that has been coated on the implanted device to facilitate the cell recruitment. Such approaches can also be applied to other medical implants in general. Alternatively, DVD-Igs can be coated on medical devices and upon implantation and releasing all DVDs from the device (or any other need which may require additional fresh DVD-Ig, including aging and denaturation of the already loaded DVD-Ig) the device could be reloaded by systemic administration of fresh DVD-Ig to the patient, where the DVD-Ig is designed to binds to a target of interest (a cytokine, a cell surface marker (such as CD34) etc.) with one set of binding sites and to a target coated on the device (including a protein, an epitope of any kind, including but not limited to lipids, polysaccharides and polymers) with the other. This technology has the advantage of extending the usefulness of coated implants.

A. Use of DVD-Igs in various diseases

DVD-Ig molecules of the invention are also useful as therapeutic molecules to treat various diseases. Such DVD molecules may bind one or more targets involved in a specific disease. Examples of such targets in various diseases are described below.

1. Human Autoimmune and Inflammatory Response

Many proteins have been implicated in general autoimmune and inflammatory responses, including C5, CCL1 (I-309), CCL11 (eotaxin), CCL13 (mcp-4), CCL15 (MIP-1d), CCL16 (HCC-4), CCL17 (TARC), CCL18 (PARC), CCL19, CCL2 (mcp-1), CCL20 (MIP-3a), CCL21 (MIP-2), CCL23 (MPIF-1), CCL24 (MPIF-2 / eotaxin-2), CCL25 (TECK), CCL26, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (mcp-3), CCL8 (mcp-2), CXCL1, CXCL10 (IP-10), CXCL11 (I-TAC / IP-9), CXCL12 (SDF1), CXCL13, CXCL14, CXCL2, CXCL3, CXCL5 (ENA-78 / LIX), CXCL6 (GCP-2), CXCL9, IL13, IL8, CCL13 (mcp-4), CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CR1, IL8RA, XCR1 (CCXCR1), IFNA2, IL10, IL13, IL17C, IL1A, IL1B, IL1F10, IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, IL22, IL5, IL8, IL9, LTA, LTB, MIF, SCYE1 (endothelial Monocyte-activating cytokine), SPP1, TNF, TNFSF5, IFNA2, IL10RA, IL10RB, IL13, IL13RA1, IL5RA, IL9, IL9R, ABCF1, BCL6, C3, C4A, CEBPB, CRP, ICEBERG, IL1R1, IL1RN, IL8RB, LTB4R, TOLLIP, FADD, IRAK1, IRAK2, MYD88, NCK2, TNFAIP3, TRADD, TRAF1, TRAF2, TRAF3, TRAF4, TRAF5, TRAF6,

ACVR1, ACVR1B, ACVR2, ACVR2B, ACVRL1, CD28, CD3E, CD3G, CD3Z, CD69, CD80, CD86, CNR1, CTLA4, CYSLTR1, FCER1A, FCER2, FCGR3A, GPR44, HAVCR2, OPRD1, P2RX7, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, BLR1, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CL1, CX3CR1, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL10, CXCL11, CXCL12, CXCL13, CXCR4, GPR2, SCYE1, SDF2, XCL1, XCL2, XCR1, AMH, AMHR2, BMPR1A, BMPR1B, BMPR2, C19orf10 (IL27w), CER1, CSF1, CSF2, CSF3, DKFZp451J0118, FGF2, GFI1, IFNA1, IFNB1, IFNG, IGF1, IL1A, IL1B, IL1R1, IL1R2, IL2, IL2RA, IL2RB, IL2RG, IL3, IL4, IL4R, IL5, IL5RA, IL6, IL6R, IL6ST, IL7, IL8, IL8RA, IL8RB, IL9, IL9R, IL10, IL10RA, IL10RB, IL11, IL11RA, IL12A, IL12B, IL12RB1, IL12RB2, IL13, IL13RA1, IL13RA2, IL15, IL15RA, IL16, IL17, IL17R, IL18, IL18R1, IL19, IL20, KITLG, LEP, LTA, LTB, LTB4R, LTB4R2, LTBR, MIF, NPPB, PDGFB, TBX21, TDGF1, TGFA, TGFB1, TGFB1I1, TGFB2, TGFB3, TGFBI, TGFBR1, TGFBR2, TGFBR3, TH1L, TNF, TNFRSF1A, TNFRSF1B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF11A, TNFRSF21, TNFSF4, TNFSF5, TNFSF6, TNFSF11, VEGF, ZFPM2, and RNF110 (ZNF144). In one aspect, DVD-Igs capable of binding one or more of the targets listed herein are provided.

2. Asthma

Allergic asthma is characterized by the presence of eosinophilia, goblet cell metaplasia, epithelial cell alterations, airway hyperreactivity (AHR), and Th2 and Th1 cytokine expression, as well as elevated serum IgE levels. It is now widely accepted that airway inflammation is the key factor underlying the pathogenesis of asthma, involving a complex interplay of inflammatory cells such as T cells, B cells, eosinophils, mast cells and macrophages, and of their secreted mediators including cytokines and chemokines. Corticosteroids are the most important anti-inflammatory treatment for asthma today, however their mechanism of action is non-specific and safety concerns exist, especially in the juvenile patient population. The development of more specific and targeted therapies is therefore warranted. There is increasing evidence that IL-13 in mice mimics many of the features of asthma, including AHR, mucus hypersecretion and airway fibrosis, independently of eosinophilic inflammation (Finotto et al., International Immunology (2005), 17(8), 993-1007; Padilla et al., Journal of Immunology (2005), 174(12), 8097-8105).

IL-13 has been implicated as having a pivotal role in causing pathological responses associated with asthma. The development of anti-IL-13 mAb therapy to reduce the effects of IL-13 in the lung is an exciting new approach that offers considerable promise as a novel treatment for asthma. However other mediators of differential immunological pathways are also involved in asthma pathogenesis, and blocking these mediators, in addition to IL-13, may offer additional therapeutic benefit. Such target pairs include, but are not limited to, IL-13 and a pro-

inflammatory cytokine, such as tumor necrosis factor-α (TNF-α). TNF-α may amplify the inflammatory response in asthma and may be linked to disease severity (McDonnell, et al., Progress in Respiratory Research (2001), 31(New Drugs for Asthma, Allergy and COPD), 247-250.). This suggests that blocking both IL-13 and TNF-α may have beneficial effects, particularly in severe airway disease. In another embodiment the DVD-Ig of the invention binds the targets IL-13 and TNFα and is used for treating asthma.

Animal models such as OVA-induced asthma mouse model, where both inflammation and AHR can be assessed, are known in the art and may be used to determine the ability of various DVD-Ig molecules to treat asthma. Animal models for studying asthma are disclosed in Coffman, et al., Journal of Experimental Medicine (2005), 201(12), 1875-1879; Lloyd, et al., Advances in Immunology (2001), 77, 263-295; Boyce et al., Journal of Experimental Medicine (2005), 201(12), 1869-1873; and Snibson, et al., Journal of the British Society for Allergy and Clinical Immunology (2005), 35(2), 146-52. In addition to routine safety assessments of these target pairs specific tests for the degree of immunosuppression may be warranted and helpful in selecting the best target pairs (see Luster et al., Toxicology (1994), 92(1-3), 229-43; Descotes, et al., Developments in biological standardization (1992), 77 99-102; Hart et al., Journal of Allergy and Clinical Immunology (2001), 108(2), 250-257).

Based on the rationale disclosed herein and using the same evaluation model for efficacy and safety other pairs of targets that DVD-Ig molecules can bind and be useful to treat asthma may be determined. In an embodiment, such targets include, but are not limited to, IL-13 and IL-1beta, since IL-1beta is also implicated in inflammatory response in asthma; IL-13 and cytokines and chemokines that are involved in inflammation, such as IL-13 and IL-9; IL-13 and IL-4; IL-13 and IL-5; IL-13 and IL-25; IL-13 and TARC; IL-13 and MDC; IL-13 and MIF; IL-13 and TGF-β; IL-13 and LHR agonist; IL-13 and CL25; IL-13 and SPRR2a; IL-13 and SPRR2b; and IL-13 and ADAM8. The present invention also provides DVD-Igs capable of binding one or more targets involved in asthma selected from the group consisting of CSF1 (MCSF), CSF2 (GM-CSF), CSF3 (GCSF), FGF2, IFNA1, IFNB1, IFNG, histamine and histamine receptors, IL1A, IL1B, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12A, IL12B, IL13, IL14, IL15, IL16, IL17, IL18, IL19, KITLG, PDGFB, IL2RA, IL4R, IL5RA, IL8RA, IL8RB, IL12RB1, IL12RB2, IL13RA1, IL13RA2, IL18R1, TSLP, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL13, CCL17, CCL18, CCL19, CCL20, CCL22, CCL24,CX3CL1, CXCL1, CXCL2, CXCL3, XCL1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CX3CR1, GPR2, XCR1, FOS, GATA3, JAK1, JAK3, STAT6, TBX21, TGFB1, TNF, TNFSF6, YY1, CYSLTR1, FCER1A, FCER2, LTB4R, TB4R2, LTBR, and Chitinase.

3. Rheumatoid arthritis

Rheumatoid arthritis (RA), a systemic disease, is characterized by a chronic inflammatory reaction in the synovium of joints and is associated with degeneration of cartilage and erosion of juxta-articular bone. Many pro-inflammatory cytokines including TNF, chemokines, and growth factors are expressed in diseased joints. Systemic administration of anti-TNF antibody or sTNFR fusion protein to mouse models of RA was shown to be anti-inflammatory and joint protective. Clinical investigations in which the activcity of TNF in RA patients was blocked with intravenously administered infliximab (Harriman G, Harper LK, Schaible TF. 1999 Summary of clinical trials in rheumatoid arthritis using infliximab, an anti-TNFalpha treatment. Ann Rheum Dis 58 Suppl 1:I61-4), a chimeric anti-TNF mAb, has provided evidence that TNF regulates IL-6, IL-8, MCP-1, and VEGF production, recruitment of immune and inflammatory cells into joints, angiogenesis, and reduction of blood levels of matrix metalloproteinases-1 and -3. A better understanding of the inflammatory pathway in rheumatoid arthritis has led to identification of other therapeutic targets involved in rheumatoid arthritis. Promising treatments such as interleukin-6 antagonists (IL-6 receptor antibody MRA, developed by Chugai, Roche (see Nishimoto, Norihiro et al., Arthritis & Rheumatism (2004), 50(6), 1761-1769), CTLA4Ig (abatacept, Genovese Mc et al 2005 Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. N Engl J Med. 353:1114-23.), and anti-B cell therapy (rituximab, Okamoto H, Kamatani N. 2004 Rituximab for rheumatoid arthritis. N Engl J Med. 351:1909) have already been tested in randomized controlled trials over the past year. Other cytokines have been identified and have been shown to be of benefit in animal models, including interleukin-15 (therapeutic antibody HuMax-IL_15, AMG 714 see Baslund, Bo et al., Arthritis & Rheumatism (2005), 52(9), 2686-2692), interleukin-17, and interleukin-18, and clinical trials of these agents are currently under way. Dual-specific antibody therapy, combining anti-TNF and another mediator, has great potential in enhancing clinical efficacy and/or patient coverage. For example, blocking both TNF and VEGF can potentially eradicate inflammation and angiogenesis, both of which are involved in pathophysiology of RA. Blocking other pairs of targets involved in RA including, but not limited to, TNF and IL-18; TNF and IL-12; TNF and IL-23; TNF and IL-1beta; TNF and MIF; TNF and IL-17; TNF and IL-15 with specific DVD Igs is also contemplated. In addition to routine safety assessments of these target pairs, specific tests for the degree of immunosuppression may be warranted and helpful in selecting the best target pairs (see Luster et al., Toxicology (1994), 92(1-3), 229-43; Descotes, et al., Developments in biological standardization (1992), 77 99-102; Hart et al., Journal of Allergy and Clinical Immunology (2001), 108(2), 250-257). Whether a DVD Ig molecule will be useful for the treatment of rheumatoid arthritis can be assessed using pre-clinical animal RA models such as the collagen-induced arthritis mouse model. Other useful models are also well known in the art (see Brand

DD., Comp Med. (2005) 55(2):114-22). Based on the cross-reactivity of the parental antibodies for human and mouse othologues (e.g.,reactivity for human and mouse TNF, human and mouse IL-15 etc.) validation studies in the mouse CIA model may be conducted with "matched surrogate antibody" derived DVD-Ig molecules; briefly, a DVD-Ig based on two (or more) mouse target specific antibodies may be matched to the extent possible to the characteristics of the parental human or humanized antibodies used for human DVD-Ig construction (similar affinity, similar neutralization potency, similar half-life etc.).

4. SLE

The immunopathogenic hallmark of SLE is the polyclonal B cell activation, which leads to hyperglobulinemia, autoantibody production and immune complex formation. The fundamental abnormality appears to be the failure of T cells to suppress the forbidden B cell clones due to generalized T cell dysregulation. In addition, B and T-cell interaction is facilitated by several cytokines such as IL-10 as well as co-stimulatory molecules such as CD40 and CD40L, B7 and CD28 and CTLA-4, which initiate the second signal. These interactions together with impaired phagocytic clearance of immune complexes and apoptotic material, perpetuate the immune response with resultant tissue injury. The following targets may be involved in SLE and can potentially be used for DVD-Ig approach for therapeutic intervention: B cell targeted therapies: CD-20, CD-22, CD-19, CD28, CD4, CD80, HLA-DRA, IL10, IL2, IL4, TNFRSF5, TNFRSF6, TNFSF5, TNFSF6, BLR1, HDAC4, HDAC5, HDAC7A, HDAC9, ICOSL, IGBP1, MS4A1, RGS1, SLA2, CD81, IFNB1, IL10, TNFRSF5, TNFRSF7, TNFSF5, AICDA, BLNK, GALNAC4S-6ST, HDAC4, HDAC5, HDAC7A, HDAC9, IL10, IL11, IL4, INHA, INHBA, KLF6, TNFRSF7, CD28, CD38, CD69, CD80, CD83, CD86, DPP4, FCER2, IL2RA, TNFRSF8, TNFSF7, CD24, CD37, CD40, CD72, CD74, CD79A, CD79B, CR2, IL1R2, ITGA2, ITGA3, MS4A1, ST6GAL1, CD1C, CHST10, HLA-A, HLA-DRA, and NT5E.; co-stimulatory signals: CTLA4 or B7.1/B7.2; inhibition of B cell survival: BlyS, BAFF; Complement inactivation: C5; Cytokine modulation: the key principle is that the net biologic response in any tissue is the result of a balance between local levels of proinflammatory or anti-inflammatory cytokines (see Sfikakis PP et al 2005 Curr Opin Rheumatol 17:550-7). SLE is considered to be a Th-2 driven disease with documented elevations in serum IL-4, IL-6, IL-10. DVD Igs capable of binding one or more targets selected from the group consisting of IL-4, IL-6, IL-10, IFN-α, and TNF-α are also contemplated. Combination of targets discussed herein will enhance therapeutic efficacy for SLE which can be tested in a number of lupus preclinical models (see Peng SL (2004) Methods Mol Med.;102:227-72). Based on the cross-reactivity of the parental antibodies for human and mouse othologues (e.g.,reactivity for human and mouse CD20, human and mouse Interferon alpha etc.) validation studies in a mouse lupus model may be conducted with "matched surrogate antibody" derived DVD-Ig molecules; briefly, a DVD-Ig based two (or more) mouse target specific

antibodies may be matched to the extent possible to the characteristics of the parental human or humanized antibodies used for human DVD-Ig construction (similar affinity, similar neutralization potency, similar half-life etc.).

5. Multiple sclerosis

Multiple sclerosis (MS) is a complex human autoimmune-type disease with a predominantly unknown etiology. Immunologic destruction of myelin basic protein (MBP) throughout the nervous system is the major pathology of multiple sclerosis. MS is a disease of complex pathologies, which involves infiltration by CD4+ and CD8+ T cells and of response within the central nervous system. Expression in the CNS of cytokines, reactive nitrogen species and costimulator molecules have all been described in MS. Of major consideration are immunological mechanisms that contribute to the development of autoimmunity. In particular, antigen expression, cytokine and leukocyte interactions, and regulatory T-cells, which help balance/modulate other T-cells such as Th1 and Th2 cells, are important areas for therapeutic target identification.

IL-12 is a proinflammatory cytokine that is produced by APC and promotes differentiation of Th1 effector cells. IL-12 is produced in the developing lesions of patients with MS as well as in EAE-affected animals. Previously it was shown that interference in IL-12 pathways effectively prevents EAE in rodents, and that in vivo neutralization of IL-12p40 using a anti-IL-12 mAb has beneficial effects in the myelin-induced EAE model in common marmosets.

TWEAK is a member of the TNF family, constitutively expressed in the central nervous system (CNS), with pro-inflammatory, proliferative or apoptotic effects depending upon cell types. Its receptor, Fn14, is expressed in CNS by endothelial cells, reactive astrocytes and neurons. TWEAK and Fn14 mRNA expression increased in spinal cord during experimental autoimmune encephalomyelitis (EAE). Anti-TWEAK antibody treatment in myelin oligodendrocyte glycoprotein (MOG) induced EAE in C57BL/6 mice resulted in a reduction of disease severity and leukocyte infiltration when mice were treated after the priming phase.

One aspect of the invention pertains to DVD Ig molecules capable of binding one or more, for example two, targets selected from the group consisting of IL-12, TWEAK, IL-23, CXCL13, CD40, CD40L, IL-18, VEGF, VLA-4, TNF, CD45RB, CD200, IFNgamma, GM-CSF, FGF, C5, CD52, and CCR2. An embodiment includes a dual-specific anti-IL-12/TWEAK DVD Ig as a therapeutic agent beneficial for the treatment of MS.

Several animal models for assessing the usefulness of the DVD molecules to treat MS are known in the art (see Steinman L, et al., (2005) Trends Immunol. 26(11):565-71; Lublin FD., et al., (1985) Springer Semin Immunopathol.8(3):197-208; Genain CP, et al., (1997) J Mol Med. 75(3):187-97; Tuohy VK, et al., (1999) J Exp Med. 189(7):1033-42; Owens T, et al., (1995)

Neurol Clin.13(1):51-73; and 't Hart BA, et al., (2005) J Immunol 175(7):4761-8. Based on the cross-reactivity of the parental antibodies for human and animal species othologues (e.g.,reactivity for human and mouse IL-12, human and mouse TWEAK etc.) validation studies in the mouse EAE model may be conducted with "matched surrogate antibody" derived DVD-Ig molecules; briefly, a DVD-Ig based on to (or more) mouse target specific antibodies may be matched to the extent possible to the characteristics of the parental human or humanized antibodies used for human DVD-Ig construction (similar affinity, similar neutralization potency, similar half-life etc.). The same concept applies to animal models in other non-rodent species, where a "matched surrogate antibody" derived DVD-Ig would be selected for the anticipated pharmacology and possibly safety studies. In addition to routine safety assessments of these target pairs specific tests for the degree of immunosuppression may be warranted and helpful in selecting the best target pairs (see Luster et al., Toxicology (1994), 92(1-3), 229-43; Descotes, et al., Developments in biological standardization (1992), 77 99-102; Jones R. 2000 Rovelizumab (ICOS Corp). IDrugs.3(4):442-6).

6. Sepsis

The pathophysiology of sepsis is initiated by the outer membrane components of both gram-negative organisms (lipopolysaccharide [LPS], lipid A, endotoxin) and gram-positive organisms (lipoteichoic acid, peptidoglycan). These outer membrane components are able to bind to the CD14 receptor on the surface of monocytes. By virtue of the recently described toll-like receptors, a signal is then transmitted to the cell, leading to the eventual production of the proinflammatory cytokines tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1). Overwhelming inflammatory and immune responses are essential features of septic shock and play a central part in the pathogenesis of tissue damage, multiple organ failure, and death induced by sepsis. Cytokines, especially tumor necrosis factor (TNF) and interleukin (IL-1), have been shown to be critical mediators of septic shock. These cytokines have a direct toxic effect on tissues; they also activate phospholipase A2. These and other effects lead to increased concentrations of platelet-activating factor, promotion of nitric oxide synthase activity, promotion of tissue infiltration by neutrophils, and promotion of neutrophil activity.

The treatment of sepsis and septic shock remains a clinical conundrum, and recent prospective trials with biological response modifiers (i.e. anti-TNF, anti-MIF) aimed at the inflammatory response have shown only modest clinical benefit. Recently, interest has shifted toward therapies aimed at reversing the accompanying periods of immune suppression. Studies in experimental animals and critically ill patients have demonstrated that increased apoptosis of lymphoid organs and some parenchymal tissues contribute to this immune suppression, anergy, and organ system dysfunction. During sepsis syndromes, lymphocyte apoptosis can be triggered by the absence of IL-2 or by the release of glucocorticoids, granzymes, or the so-called 'death'

cytokines: tumor necrosis factor alpha or Fas ligand. Apoptosis proceeds via auto-activation of cytosolic and/or mitochondrial caspases, which can be influenced by the pro- and anti-apoptotic members of the Bcl-2 family. In experimental animals, not only can treatment with inhibitors of apoptosis prevent lymphoid cell apoptosis; it may also improve outcome. Although clinical trials with anti-apoptotic agents remain distant due in large part to technical difficulties associated with their administration and tissue targeting, inhibition of lymphocyte apoptosis represents an attractive therapeutic target for the septic patient. Likewise, a dual-specific agent targeting both inflammatory mediator and a apoptotic mediator, may have added benefit. One aspect of the invention pertains to DVD Igs capable of binding one or more targets involved in sepsis, in an embodiment two targets, selected from the group consisting TNF, IL-1, MIF, IL-6, IL-8, IL-18, IL-12, IL-23, FasL, LPS, Toll-like receptors, TLR-4, tissue factor, MIP-2, ADORA2A, CASP1, CASP4, IL-10, IL-1B, NFKB1, PROC, TNFRSF1A, CSF3, CCR3, IL1RN, MIF, NFKB1, PTAFR, TLR2, TLR4, GPR44, HMOX1, midkine, IRAK1, NFKB2, SERPINA1, SERPINE1, and TREM1. The efficacy of such DVD Igs for sepsis can be assessed in preclinical animal models known in the art (see Buras JA, et al.,(2005) Nat Rev Drug Discov. 4(10):854-65 and Calandra T, et al., (2000) Nat Med. 6(2):164-70).

7. Neurological disorders

7.1. Neurodegenerative Diseases

Chronic neurodegenerative diseases are usually age-dependent diseases characterized by progressive loss of neuronal functions (neuronal cell death, demyelination), loss of mobility and loss of memory. Emerging knowledge of the mechanisms underlying chronic neurodegenerative diseases (e.g., Alzheimer's disease disease) show a complex etiology and a variety of factors have been recognized to contribute to their development and progression e.g.,age, glycemic status, amyloid production and multimerization, accumulation of advanced glycation-end products (AGE) which bind to their receptor RAGE (receptor for AGE), increased brain oxidative stress, decreased cerebral blood flow, neuroinflammation including release of inflammatory cytokines and chemokines, neuronal dysfunction and microglial activation. Thus these chronic neurodegenerative diseases represent a complex interaction between multiple cell types and mediators. Treatment strategies for such diseases are limited and mostly constitute either blocking inflammatory processes with non-specific anti-inflammatory agents (e.g., corticosteroids, COX inhibitors) or agents to prevent neuron loss and/or synaptic functions. These treatments fail to stop disease progression. Recent studies suggest that more targeted therapies such as antibodies to soluble A-b peptide (including the A-b oligomeric forms) can not only help stop disease progression but may help maintain memory as well. These preliminary observations suggest that specific therapies targeting more than one disease mediator (e.g.,A-b and a pro-inflammatory cytokine such as TNF) may provide even better therapeutic efficacy for

chronic neurodegenerative diseases than observed with targeting a single disease mechanism (e.g.,soluble A-balone) (see C.E. Shepherd, et al, Neurobiol Aging. 2005 Oct 24; Nelson RB., Curr Pharm Des. 2005;11:3335; William L. Klein.; Neurochem Int. 2002 ;41:345; Michelle C Janelsins, et al., J Neuroinflammation. 2005 ;2:23; Soloman B., Curr Alzheimer Res. 2004;1:149; Igor Klyubin, et al., Nat Med. 2005;11:556-61; Arancio O, et al., EMBO Journal (2004) 1-10; Bornemann KD, et al., Am J Pathol. 2001;158:63; Deane R, et al., Nat Med. 2003;9:907-13; and Eliezer Masliah, et al., Neuron. 2005;46:857).

The DVD-Ig molecules of the invention can bind one or more targets involved in Chronic neurodegenerative diseases such as Alzheimers. Such targets include, but are not limited to, any mediator, soluble or cell surface, implicated in AD pathogenesis e.g AGE (S100 A, amphoterin), pro-inflammatory cytokines (e.g.,IL-1), chemokines (e.g.,MCP 1), molecules that inhibit nerve regeneration (e.g.,Nogo, RGM A), molecules that enhance neurite growth (neurotrophins). The efficacy of DVD-Ig molecules can be validated in pre-clinical animal models such as the transgenic mice that over-express amyloid precursor protein or RAGE and develop Alzheimer's disease-like symptoms. In addition, DVD-Ig molecules can be constructed and tested for efficacy in the animal models and the best therapeutic DVD-Ig can be selected for testing in human patients. DVD-Ig molecules can also be employed for treatment of other neurodegenerative diseases such as Parkinson's disease. Alpha-Synuclein is involved in Parkinson's pathology. A DVD-Ig capable of targeting alpha-synuclein and inflammatory mediators such as TNF, IL-1, MCP-1 can prove effective therapy for Parkinson's disease and are contemplated in the invention.

7.2 Neuronal Regeneration and Spinal Cord Injury

Despite an increase in knowledge of the pathologic mechanisms, spinal cord injury (SCI) is still a devastating condition and represents a medical indication characterized by a high medical need. Most spinal cord injuries are contusion or compression injuries and the primary injury is usually followed by secondary injury mechanisms (inflammatory mediators e.g.,cytokines and chemokines) that worsen the initial injury and result in significant enlargement of the lesion area, sometimes more than 10-fold. These primary and secondary mechanisms in SCI are very similar to those in brain injury caused by other means e.g.,stroke. No satisfying treatment exists and high dose bolus injection of methylprednisolone (MP) is the only used therapy within a narrow time window of 8 h post injury. This treatment, however, is only intended to prevent secondary injury without causing any significant functional recovery. It is heavily critisized for the lack of unequivocal efficacy and severe adverse effects, like immunosuppression with subsequent infections and severe histopathological muscle alterations. No other drugs, biologics or small molecules, stimulating the endogenous regenerative potential are approved, but promising treatment principles and drug candidates have shown efficacy in animal models of SCI in recent

years. To a large extent the lack of functional recovery in human SCI is caused by factors inhibiting neurite growth, at lesion sites, in scar tissue, in myelin as well as on injury-associated cells. Such factors are the myelin-associated proteins NogoA, OMgp and MAG, RGM A, the scar-associated CSPG (Chondroitin Sulfate Proteoglycans) and inhibitory factors on reactive astrocytes (some semaphorins and ephrins). However, at the lesion site not only growth inhibitory molecules are found but also neurite growth stimulating factors like neurotrophins, laminin, L1 and others. This ensemble of neurite growth inhibitory and growth promoting molecules may explain that blocking single factors, like NogoA or RGM A, resulted in significant functional recovery in rodent SCI models, because a reduction of the inhibitory influences could shift the balance from growth inhibition to growth promotion. However, recoveries observed with blocking a single neurite outgrowth inhibitory molecule were not complete. To achieve faster and more pronounced recoveries either blocking two neurite outgrowth inhibitory molecules e.g Nogo and RGM A, or blocking an neurite outgrowth inhibitory molecule and enhancing functions of a neurite outgrowth enhancing molecule e.g Nogo and neurotrophins, or blocking a neurite outgrowth inhibitory moleclule e.g.,Nogo and a pro-inflammatory molecule e.g.,TNF, may be desirable (see McGee AW, et al., Trends Neurosci. 2003;26:193; Marco Domeniconi, et al., J Neurol Sci. 2005;233:43; Milan Makwanal, et al., FEBS J. 2005;272:2628; Barry J. Dickson, Science. 2002;298:1959; Felicia Yu Hsuan Teng, et al., J Neurosci Res. 2005;79:273; Tara Karnezis, et al., Nature Neuroscience 2004; 7, 736; Gang Xu, et al., J. Neurochem.2004; 91; 1018).

In one aspect, DVD-Igs capable of binding target pairs such as NgR and RGM A; NogoA and RGM A; MAG and RGM A; OMGp and RGM A; RGM A and RGM B; CSPGs and RGM A; aggrecan, midkine, neurocan, versican, phosphacan, Te38 and TNF-α; Aß globulomer-specific antibodies combined with antibodies promoting dendrite & axon sprouting are provided. Dendrite pathology is a very early sign of AD and it is known that NOGO A restricts dendrite growth. One can combine such type of ab with any of the SCI-candidate (myelin-proteins) Ab. Other DVD-Ig targets may include any combination of NgR-p75, NgR-Troy, NgR-Nogo66 (Nogo), NgR-Lingo, Lingo-Troy, Lingo-p75, MAG or Omgp. Additionally, targets may also include any mediator, soluble or cell surface, implicated in inhibition of neurite e.g Nogo, Ompg, MAG, RGM A, semaphorins, ephrins, soluble A-b, pro-inflammatory cytokines (e.g.,IL-1), chemokines (e.g.,MIP 1a), molecules that inhibit nerve regeneration. The efficacy of anti-nogo / anti-RGM A or similar DVD-Ig molecules can be validated in pre-clinical animal models of spinal cord injury. In addition, these DVD-Ig molecules can be constructed and tested for efficacy in the animal models and the best therapeutic DVD-Ig can be selected for testing in human patients. In addition, DVD-Ig molecules can be constructed that target two distinct ligand binding sites on a single receptor e.g.,Nogo receptor which binds three ligand Nogo, Ompg, and

MAG and RAGE that binds A-b and S100 A. Furthermore, neurite outgrowth inihibitors e.g.,nogo and nogo receptor, also play a role in preventing nerve regeneration in immunological diseases like multiple sclerosis. Inhibition of nogo-nogo receptor interaction has been shown to enhance recovery in animal models of multiple sclerosis. Therefore, DVD-Ig molecules that can block the function of one immune mediator eg a cytokine like IL-12 and a neurite outgrowth inhibitor molecule eg nogo or RGM may offer faster and greater efficacy than blocking either an immune or an neurite outgrowth inhibitor molecule alone.

8. Oncological disorders

Monoclonal antibody therapy has emerged as an important therapeutic modality for cancer (von Mehren M, et al 2003 Monoclonal antibody therapy for cancer. Annu Rev Med.;54:343-69). Antibodies may exert antitumor effects by inducing apoptosis, redirected cytotoxicity, interfering with ligand-receptor interactions, or preventing the expression of proteins that are critical to the neoplastic phenotype. In addition, antibodies can target components of the tumor microenvironment, perturbing vital structures such as the formation of tumor-associated vasculature. Antibodies can also target receptors whose ligands are growth factors, such as the epidermal growth factor receptor. The antibody thus inhibits natural ligands that stimulate cell growth from binding to targeted tumor cells. Alternatively, antibodies may induce an anti-idiotype network, complement-mediated cytotoxicity, or antibody-dependent cellular cytotoxicity (ADCC). The use of dual-specific antibody that targets two separate tumor mediators will likely give additional benefit compared to a mono-specific therapy. DVD Igs capable of binding the following pairs of targets to treat oncological disease are also contemplated: IGF1 and IGF2; IGF1/2 and HER-2; VEGFR and EGFR; CD20 and CD3; CD138 and CD20; CD38 and CD20; CD38 and CD138; CD40 and CD20; CD138 and CD40; CD38 and CD40; CD-20 and CD-19; CD-20 and EGFR; CD-20 and CD-80; CD-20 and CD-22; CD-3 and HER-2; CD-3 and CD-19; EGFR and HER-2; EGFR and CD-3; EGFR and IGF1,2; EGFR and IGF1R; EGFR and RON; EGFR and HGF; EGFR and c-MET; HER-2 and IGF1,2; HER-2 and IGF1R; RON and HGF; VEGF and EGFR; VEGF and HER-2; VEGF and CD-20; VEGF and IGF1,2; VEGF and DLL4; VEGF and HGF; VEGF and RON; VEGF and NRP1; CD20 and CD3; VEGF and PLGF; DLL4 and PLGF; ErbB3 and EGFR; HGF and ErbB3, HER-2 and ErbB3; c-Met and ErbB3; HER-2 and PLGF; HER-2 and HER-2; EGFR and EGFR; EGFR and DLL-4; EGFR and PLGF; EGFR and RGMa; EGFR and tetanus toxoid; VEGF and tetanus toxoid; and tetanus toxoid and tetanus toxoid.

In another embodiment, a DVD of the invention is capable of binding VEGF and phosphatidylserine; VEGF and ErbB3; VEGF and PLGF; VEGF and ROBO4; VEGF and BSG2; VEGF and CDCP1; VEGF and ANPEP; VEGF and c-MET; HER-2 and ERB3; HER-2 and BSG2; HER-2 and CDCP1; HER-2 and ANPEP; EGFR and CD64; EGFR and BSG2; EGFR and

CDCP1; EGFR and ANPEP; IGF1R and PDGFR; IGF1R and VEGF; IGF1R and CD20; CD20 and CD74; CD20 and CD30; CD20 and DR4; CD20 and VEGFR2; CD20 and CD52; CD20 and CD4; HGF and c-MET; HGF and NRP1; HGF and phosphatidylserine; ErbB3 and IGF1R; ErbB3 and IGF1,2; c-Met and Her-2; c-Met and NRP1; c-Met and IGF1R; IGF1,2 and PDGFR; IGF1,2 and CD20; IGF1,2 and IGF1R; IGF2 and EGFR; IGF2 and HER2; IGF2 and CD20; IGF2 and VEGF; IGF2 and IGF1R; IGF1 and IGF2; PDGFRa and VEGFR2; PDGFRa and PLGF; PDGFRa and VEGF; PDGFRa and c-Met; PDGFRa and EGFR; PDGFRb and VEGFR2; PDGFRb and c-Met; PDGFRb and EGFR; RON and c-Met; RON and MTSP1; RON and MSP; RON and CDCP1; VGFR1 and PLGF; VGFR1 and RON; VGFR1 and EGFR; VEGFR2 and PLGF; VEGFR2 and NRP1; VEGFR2 and RON; VEGFR2 and DLL4; VEGFR2 and EGFR; VEGFR2 and ROBO4; VEGFR2 and CD55; LPA and S1P; EPHB2 and RON; CTLA4 and VEGF; CD3 and EPCAM; CD40 and IL6; CD40 and IGF; CD40 and CD56; CD40 and CD70; CD40 and VEGFR1; CD40 and DR5; CD40 and DR4; CD40 and APRIL; CD40 and BCMA; CD40 and RANKL; CD28 and MAPG; CD80 and CD40; CD80 and CD30; CD80 and CD33; CD80 and CD74; CD80 and CD2; CD80 and CD3; CD80 and CD19; CD80 and CD4; CD80 and CD52; CD80 and VEGF; CD80 and DR5; CD80 and VEGFR2; CD22 and CD20; CD22 and CD80; CD22 and CD40; CD22 and CD23; CD22 and CD33; CD22 and CD74; CD22 and CD19; CD22 and DR5; CD22 and DR4; CD22 and VEGF; CD22 and CD52; CD30 and CD20; CD30 and CD22; CD30 and CD23; CD30 and CD40; CD30 and VEGF; CD30 and CD74; CD30 and CD19; CD30 and DR5; CD30 and DR4; CD30 and VEGFR2; CD30 and CD52; CD30 and CD4; CD138 and RANKL; CD33 and FTL3; CD33 and VEGF; CD33 and VEGFR2; CD33 and CD44; CD33 and DR4; CD33 and DR5; DR4 and CD137; DR4 and IGF1,2; DR4 and IGF1R; DR4 and DR5; DR5 and CD40; DR5 and CD137; DR5 and CD20; DR5 and EGFR; DR5 and IGF1,2; DR5 and IGFR, DR5 and HER-2, EGFR and DLL4. Other target combinations include one or more members of the EGF/erb-2/erb-3 family. Other targets (one or more) involved in oncological diseases that DVD Igs may bind include, but are not limited to those selected from the group consisting of: CD52, CD20, CD19, CD3, CD4, CD8, BMP6, IL12A, IL1A, IL1B, IL2, IL24, INHA, TNF, TNFSF10, BMP6, EGF, FGF1, FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GRP, IGF1, IGF2, IL12A, IL1A, IL1B, IL2, INHA, TGFA, TGFB1, TGFB2, TGFB3, VEGF, CDK2, FGF10, FGF18, FGF2, FGF4, FGF7, IGF1R, IL2, BCL2, CD164, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CDKN3, GNRH1, IGFBP6, IL1A, IL1B, ODZ1, PAWR, PLG, TGFB1I1, AR, BRCA1, CDK3, CDK4, CDK5, CDK6, CDK7, CDK9, E2F1, EGFR, ENO1, ERBB2, ESR1, ESR2, IGFBP3, IGFBP6, IL2, INSL4, MYC, NOX5, NR6A1, PAP, PCNA, PRKCQ, PRKD1, PRL, TP53, FGF22, FGF23, FGF9, IGFBP3, IL2, INHA, KLK6, TP53, CHGB, GNRH1, IGF1, IGF2, INHA, INSL3, INSL4, PRL, KLK6, SHBG, NR1D1, NR1H3, NR1I3, NR2F6, NR4A3, ESR1, ESR2, NR0B1, NR0B2,

NR1D2, NR1H2, NR1H4, NR1I2, NR2C1, NR2C2, NR2E1, NR2E3, NR2F1, NR2F2, NR3C1, NR3C2, NR4A1, NR4A2, NR5A1, NR5A2, NR6A1, PGR, RARB, FGF1, FGF2, FGF6, KLK3, KRT1, APOC1, BRCA1, CHGA, CHGB, CLU, COL1A1, COL6A1, EGF, ERBB2, ERK8, FGF1, FGF10, FGF11, FGF13, FGF14, FGF16, FGF17, FGF18, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GNRH1, IGF1, IGF2, IGFBP3, IGFBP6, IL12A, IL1A, IL1B, IL2, IL24, INHA, INSL3, INSL4, KLK10, KLK12, KLK13, KLK14, KLK15, KLK3, KLK4, KLK5, KLK6, KLK9, MMP2, MMP9, MSMB, NTN4, ODZ1, PAP, PLAU, PRL, PSAP, SERPINA3, SHBG, TGFA, TIMP3, CD44, CDH1, CDH10, CDH19, CDH20, CDH7, CDH9, CDH1, CDH10, CDH13, CDH18, CDH19, CDH20, CDH7, CDH8, CDH9, ROBO2, CD44, ILK, ITGA1, APC, CD164, COL6A1, MTSS1, PAP, TGFB1I1, AGR2, AIG1, AKAP1, AKAP2, CANT1, CAV1, CDH12, CLDN3, CLN3, CYB5, CYC1, DAB2IP, DES, DNCL1, ELAC2, ENO2, ENO3, FASN, FLJ12584, FLJ25530, GAGEB1, GAGEC1, GGT1, GSTP1, HIP1, HUMCYT2A, IL29, K6HF, KAI1, KRT2A, MIB1, PART1, PATE, PCA3, PIAS2, PIK3CG, PPID, PR1, PSCA, SLC2A2, SLC33A1, SLC43A1, STEAP, STEAP2, TPM1, TPM2, TRPC6, ANGPT1, ANGPT2, ANPEP, ECGF1, EREG, FGF1, FGF2, FIGF, FLT1, JAG1, KDR, LAMA5, NRP1, NRP2, PGF, PLXDC1, STAB1, VEGF, VEGFC, ANGPTL3, BAI1, COL4A3, IL8, LAMA5, NRP1, NRP2, STAB1, ANGPTL4, PECAM1, PF4, PROK2, SERPINF1, TNFAIP2, CCL11, CCL2, CXCL1, CXCL10, CXCL3, CXCL5, CXCL6, CXCL9, IFNA1, IFNB1, IFNG, IL1B, IL6, MDK, EDG1, EFNA1, EFNA3, EFNB2, EGF, EPHB4, FGFR3, HGF, IGF1, ITGB3, PDGFA, TEK, TGFA, TGFB1, TGFB2, TGFBR1, CCL2, CDH5, COL18A1, EDG1, ENG, ITGAV, ITGB3, THBS1, THBS2, BAD, BAG1, BCL2, CCNA1, CCNA2, CCND1, CCNE1, CCNE2, CDH1 (E-cadherin), CDKN1B (p27Kip1), CDKN2A (p16INK4a), COL6A1, CTNNB1 (b-catenin), CTSB (cathepsin B), ERBB2 (Her-2), ESR1, ESR2, F3 (TF), FOSL1 (FRA-1), GATA3, GSN (Gelsolin), IGFBP2, IL2RA, IL6, IL6R, IL6ST (glycoprotein 130), ITGA6 (a6 integrin), JUN, KLK5, KRT19, MAP2K7 (c-Jun), MKI67 (Ki-67), NGFB (NGF), NGFR, NME1 (NM23A), PGR, PLAU (uPA), PTEN, SERPINB5 (maspin), SERPINE1 (PAI-1), TGFA, THBS1 (thrombospondin-1), TIE (Tie-1), TNFRSF6 (Fas), TNFSF6 (FasL), TOP2A (topoisomerase Iia), TP53, AZGP1 (zinc-a-glycoprotein), BPAG1 (plectin), CDKN1A (p21Wap1/Cip1), CLDN7 (claudin-7), CLU (clusterin), ERBB2 (Her-2), FGF1, FLRT1 (fibronectin), GABRP (GABAa), GNAS1, ID2, ITGA6 (a6 integrin), ITGB4 (b 4 integrin), KLF5 (GC Box BP), KRT19 (Keratin 19), KRTHB6 (hair-specific type II keratin), MACMARCKS, MT3 (metallothionectin-III), MUC1 (mucin), PTGS2 (COX-2), RAC2 (p21Rac2), S100A2, SCGB1D2 (lipophilin B), SCGB2A1 (mammaglobin 2), SCGB2A2 (mammaglobin 1), SPRR1B (Spr1), THBS1, THBS2, THBS4, and TNFAIP2 (B94), RON, c-Met, CD64, DLL4, PLGF, CTLA4, phophatidylserine, ROBO4, CD80, CD22, CD40, CD23, CD28, CD80, CD55, CD38, CD70, CD74, CD30, CD138, CD56, CD33, CD2, CD137, DR4, DR5, RANKL, VEGFR2, PDGFR, VEGFR1, MTSP1, MSP, EPHB2, EPHA1, EPHA2, EpCAM,

PGE2, NKG2D, LPA, SIP, APRIL, BCMA, MAPG, FLT3, PDGFR alpha, PDGFR beta, ROR1, PSMA, PSCA, SCD1, and CD59.

IV. Pharmaceutical Composition

The invention also provides pharmaceutical compositions comprising a binding protein, of the invention and a pharmaceutically acceptable carrier. The pharmaceutical compositions comprising binding proteins of the invention are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, managing, or ameliorating of a disorder or one or more symptoms thereof, and/or in research. In a specific embodiment, a composition comprises one or more binding proteins of the invention. In another embodiment, the pharmaceutical composition comprises one or more binding proteins of the invention and one or more prophylactic or therapeutic agents other than binding proteins of the invention for treating a disorder. In an embodiment, the prophylactic or therapeutic agents known to be useful for or having been or currently being used in the prevention, treatment, management, or amelioration of a disorder or one or more symptoms thereof. In accordance with these embodiments, the composition may further comprise of a carrier, diluent or excipient.

The binding proteins of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises a binding protein of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In some embodiments, isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride, are included in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

Various delivery systems are known and can be used to administer one or more antibodies of the invention or the combination of one or more antibodies of the invention and a prophylactic agent or therapeutic agent useful for preventing, managing, treating, or ameliorating a disorder or one or more symptoms thereof, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor- mediated endocytosis (see, e. g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering a prophylactic or therapeutic agent of the invention include, but are not limited to, parenteral administration (e.g.,

intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous) , epidurala administration, intratumoral administration, and mucosal adminsitration (e.g., intranasal and oral routes). In addition, pulmonary administration can be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference their entireties. In one embodiment, a binding protein of the invention, combination therapy, or a composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.). In a specific embodiment, prophylactic or therapeutic agents of the invention are administered intramuscularly, intravenously, intratumorally, orally, intranasally, pulmonary, or subcutaneously. The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

In a specific embodiment, it may be desirable to administer the prophylactic or therapeutic agents of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous or non-porous material, including membranes and matrices, such as sialastic membranes, polymers, fibrous matrices (e.g., Tissuel®), or collagen matrices. In one embodiment, an effective amount of one or more antibodies of the invention antagonists is administered locally to the affected area to a subject to prevent, treat, manage, and/or ameliorate a disorder or a symptom thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered locally to the affected area in combination with an effective amount of one or more therapies (e. g., one or more prophylactic or therapeutic agents) other than a binding protein of the invention of a subject to prevent, treat, manage, and/or ameliorate a disorder or one or more symptoms thereof.

In another embodiment, the prophylactic or therapeutic agent can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:20; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the therapies of the invention (see e.g., Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York

(1984); Ranger and Peppas, 1983, J., Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 7 1:105); U.S. Pat. No. 5,679,377; U.S. Pat. No. 5, 916,597; U. S. Pat. No. 5,912,015; U.S. Pat. No. 5,989,463; U.S. Pat. No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N- vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In an embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. See, e.g., U. S. Pat. No. 4,526, 938, PCT publication WO 91/05548, PCT publication WO 96/20698, Ning et al. , 1996, "Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," Radiotherapy &Oncology 39:179-189, Song et al., 1995, "Antibody Mediated Lung Targeting of Long- Circulating Emulsions," PDA Journal of Pharmaceutical Science &Technology 50:372-397, Cleek et al., 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, and Lam et al., 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759- 760, each of which is incorporated herein by reference in their entireties.

In a specific embodiment, where the composition of the invention is a nucleic acid encoding a prophylactic or therapeutic agent, the nucleic acid can be administered in vivo to promote expression of its encoded prophylactic or therapeutic agent, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U. S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868). Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, oral, intranasal (e.g., inhalation), transdermal (e.g., topical), transmucosal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocamne to ease pain at the site of the injection.

If the compositions of the invention are to be administered topically, the compositions can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, Pa. (1995). In an embodiment, for non- sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity greater than water are employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, in an embodiment, in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (e.g., a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known in the art.

If the method of the invention comprises intranasal administration of a composition, the composition can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, e.g., gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

If the method of the invention comprises oral administration, compositions can be formulated orally in the form of tablets, capsules, cachets, gelcaps, solutions, suspensions, and the like. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate) ; lubricants (e.g., magnesium stearate, talc, or silica); disintegrants (e.g., potato starch or sodium starch glycolate) ; or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, but not limited to, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p- hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of a prophylactic or therapeutic agent(s).

The method of the invention may comprise pulmonary administration, e.g., by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference their entireties. In a specific embodiment, a binding protein of the invention, combination therapy, and/or composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.).

The method of the invention may comprise administration of a composition formulated for parenteral administration by injection (e. g., by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form (e.g., in ampoules or in multi-dose containers) with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (e.g., sterile pyrogen-free water) before use.

The methods of the invention may additionally comprise of administration of compositions formulated as depot preparations. Such long acting formulations may be

administered by implantation (e.g., subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compositions may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives (e.g., as a sparingly soluble salt).

The methods of the invention encompasse administration of compositions formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2- ethylamino ethanol, histidine, procaine, etc.

Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the mode of administration is infusion, composition can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the mode of administration is by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

In particular, the invention also provides that one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the agent. In one embodiment, one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject. In an embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, or at least 100 mg. The lyophilized prophylactic or therapeutic agents or pharmaceutical compositions of the invention should be stored at between 2° C. and 8° C. in its original container and the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention should be administered within 1 week, e.g., within 5 days, within 72 hours, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the agent. In an embodiment, the liquid form of the administered composition is supplied in a hermetically sealed container at least 0.25 mg/ml, at least 0.5 mg/ml, at least 1 mg/ml, at least 2.5

mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml or at least 100 mg/ml. The liquid form should be stored at between 2° C. and 8° C. in its original container.

The binding proteins of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. In an embodiment, the antibody or antibody-portions will be prepared as an injectable solution containing 0.1-250 mg/ml binding protein. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants. The pharmaceutical composition comprising the binding proteins of the invention prepared as an injectable solution for parenteral administration, can further comprise an agent useful as an adjuvant, such as those used to increase the absorption, or dispersion of a therapeutic protein (e.g., antibody). A particularly useful adjuvant is hyaluronidase, such as Hylenex® (recombinant human hyaluronidase). Addition of hyaluronidase in the injectable solution improves human bioavailability following parenteral administration, particularly subcutaneous administration. It also allows for greater injection site volumes (i.e. greater than 1 ml) with less pain and discomfort, and minimum incidence of injection site reactions. (see WO2004078140, and US2006104968 incorporated herein by reference).

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The form chosen depends on the intended mode of administration and therapeutic application. Typical compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The chosen mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In an embodiment, the antibody is administered by intravenous infusion or

injection. In another embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (*i.e.*, antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, the methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including, in the composition, an agent that delays absorption, for example, monostearate salts and gelatin.

The binding proteins of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, in an embodiment, the route/mode of administration is subcutaneous injection, intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, a binding protein of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer

a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, a binding protein of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating disorders with binding protein of the invention. For example, a binding protein of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (*e.g.*, antibodies that bind other cytokines or that bind cell surface molecules). Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

In certain embodiments, a binding protein is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Such vehicles are described, e.g., in U.S. Application Serial No. 09/428,082 and published PCT Application No. WO 99/25044, which are hereby incorporated by reference for any purpose.

In a specific embodiment, nucleic acid sequences encoding a binding protein of the invention or another prophylactic or therapeutic agent of the invention are administered to treat, prevent, manage, or ameliorate a disorder or one or more symptoms thereof by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded antibody or prophylactic or therapeutic agent of the invention that mediates a prophylactic or therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, Science 260:926- 932 (1993); and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley &Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990). Detailed description of various methods of gene therapy are disclosed in US20050042664 A1 which is incorporated herein by reference.

The binding proteins of the invention are useful in treating various diseases wherein the targets that are recognized by the binding proteins are detrimental. Such diseases include, but are not limited to, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthopathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthopathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated

hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasulitis of the kidneys, lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjörgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (*Hashimoto's disease*), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatatis, idiosyncratic liver disease, Drug-Induced hepatitis, Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (*e.g.*, depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), Abetalipoprotemia, Acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-l- antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti cd3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aordic and peripheral aneuryisms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, Burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chromic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, Dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis,

dermatologic conditions, diabetes, diabetes mellitus, diabetic ateriosclerotic disease, Diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's Syndrome in middle age, drug- induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, epstein-barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hematophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallerrorden-Spatz disease, hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, *hepatitis* (A), *His bundle arrythmias, HIV infection/HIV neuropathy, Hodgkin's disease,* hyperkinetic movement disorders, hypersensitity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, Asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza a, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia- reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant rejection, lymphederma, malaria, malignamt Lymphoma, malignant histiocytosis, malignant melanoma, meningitis, meningococcemia, metabolic/idiopathic, migraine headache, mitochondrial multi.system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel Dejerine- Thomas Shi-Drager and Machado-Joseph), myasthenia gravis, mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodyplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic I muscular atrophies , neutropenic fever, non-hodgkins lymphoma, occlusion of the abdominal aorta and its branches, occulsive arterial disorders, okt3 therapy, orchitis/epidydimitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherlosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiotomy syndrome, preeclampsia, Progressive supranucleo Palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynoud's disease, Refsum's disease,

regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, Subacute sclerosing panencephalitis, Syncope, syphilis of the cardiovascular system, systemic anaphalaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, ,vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke- Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue. (see Peritt et al. PCT publication No. WO2002097048A2, Leonard et al., PCT publication No. WO9524918 A1, and Salfeld et al., PCT publication No. WO00/56772A1).

The binding proteins of the invention can be used to treat humans suffering from autoimmune diseases, in particular those associated with inflammation, including, rheumatoid arthritis, spondylitis, allergy, autoimmune diabetes, autoimmune uveitis. In an embodiment, the binding proteins of the invention or antigen-binding portions thereof, are used to treat rheumatoid arthritis, Crohn's disease, multiple sclerosis, insulin dependent diabetes mellitus and psoriasis.

In an embodiment, diseases that can be treated or diagnosed with the compositions and methods of the invention include, but are not limited to, primary and metastatic cancers, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma), tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas), solid tumors arising from hematopoietic malignancies such as leukemias, and lymphomas (both Hodgkin's and non-Hodgkin's lymphomas).

In an embodiment, the antibodies of the invention or antigen-binding portions thereof, are used to treat cancer or in the prevention of metastases from the tumors described herein either when used alone or in combination with radiotherapy and/or other chemotherapeutic agents.

The antibodies of the invention, or antigen binding portions thereof, may be combined with agents that include but are not limited to, antineoplastic agents, radiotherapy, chemotherapy such as DNA alkylating agents, cisplatin, carboplatin, anti-tubulin agents, paclitaxel, docetaxel, taxol, doxorubicin, gemcitabine, gemzar, anthracyclines, adriamycin, topoisomerase I inhibitors, topoisomerase II inhibitors, 5-fluorouracil (5-FU), leucovorin, irinotecan, receptor tyrosine kinase inhibitors (e.g., erlotinib, gefitinib), COX-2 inhibitors (e.g., celecoxib), kinase inhibitors, and siRNAs.

A binding protein of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of various diseases.

A binding protein of the invention can be used alone or in combination to treat such diseases. It should be understood that the binding proteins can be used alone or in combination with an additional agent, e.g., a therapeutic agent, said additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the antibody of the present invention. The additional agent also can be an agent that imparts a beneficial attribute to the therapeutic composition e.g., an agent which effects the viscosity of the composition.

It should further be understood that the combinations which are to be included within this invention are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations, which are part of this invention, can be the antibodies of the present invention and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

Combinations to treat autoimmune and inflammatory diseases are non-steroidal anti-inflammatory drug(s) also referred to as NSAIDS which include drugs like ibuprofen. Other combinations are corticosteroids including prednisolone; the well known side-effects of steroid use can be reduced or even eliminated by tapering the steroid dose required when treating patients in combination with the DVD Igs of this invention. Non-limiting examples of therapeutic agents for rheumatoid arthritis with which an antibody, or antibody portion, of the invention can be combined include the following: cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, IL-21, IL-23, interferons, EMAP-II, GM-CSF, FGF, and PDGF. Binding proteins of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3,

CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, CTLA or their ligands including CD154 (gp39 or CD40L).

Combinations of therapeutic agents may interfere at different points in the autoimmune and subsequent inflammatory cascade; examples include TNF antagonists like chimeric, humanized or human TNF antibodies, ADALIMUMAB, (PCT Publication No. WO 97/29131), CA2 (Remicade™), CDP 571, and soluble p55 or p75 TNF receptors, derivatives, thereof, (p75TNFR1gG (Enbrel™) or p55TNFR1gG (Lenercept), and also TNFα converting enzyme (TACE) inhibitors; similarly IL-1 inhibitors (Interleukin-1-converting enzyme inhibitors, IL-1RA etc.) may be effective for the same reason. Other combinations include Interleukin 11. Yet another combination include key players of the autoimmune response which may act parallel to, dependent on or in concert with IL-12 function; especially are IL-18 antagonists including IL-18 antibodies or soluble IL-18 receptors, or IL-18 binding proteins. It has been shown that IL-12 and IL-18 have overlapping but distinct functions and a combination of antagonists to both may be most effective. Yet another combination are non-depleting anti-CD4 inhibitors. Yet other combinations include antagonists of the co-stimulatory pathway CD80 (B7.1) or CD86 (B7.2) including antibodies, soluble receptors or antagonistic ligands.

The binding proteins of the invention may also be combined with agents, such as methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine chloroquinine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, cochicine, corticosteroids (oral, inhaled and local injection), beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral), xanthines (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium and oxitropium, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adensosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNF-αor IL-1 (e.g.,IRAK, NIK, IKK , p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TNFαconverting enzyme (TACE) inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g.,soluble p55 or p75 TNF receptors and the derivatives p75TNFRIgG (Enbrel™ and p55TNFRIgG (Lenercept)), sIL-1RI, sIL-1RII, sIL-6R), antiinflammatory cytokines (e.g.,IL-4, IL-10, IL-11, IL-13 and TGFβ), celecoxib, folic acid, hydroxychloroquine sulfate, rofecoxib, etanercept, infliximab, naproxen, valdecoxib, sulfasalazine, methylprednisolone, meloxicam, methylprednisolone acetate, gold sodium thiomalate, aspirin, triamcinolone acetonide, propoxyphene napsylate/apap, folate, nabumetone, diclofenac, piroxicam, etodolac, diclofenac sodium, oxaprozin, oxycodone hcl, hydrocodone

bitartrate/apap, diclofenac sodium/misoprostol, fentanyl, anakinra, human recombinant, tramadol hcl, salsalate, sulindac, cyanocobalamin/fa/pyridoxine, acetaminophen, alendronate sodium, prednisolone, morphine sulfate, lidocaine hydrochloride, indomethacin, glucosamine sulf/chondroitin, amitriptyline hcl, sulfadiazine, oxycodone hcl/acetaminophen, olopatadine hcl, misoprostol, naproxen sodium, omeprazole, cyclophosphamide, rituximab, IL-1 TRAP, MRA, CTLA4-IG, IL-18 BP, anti-IL-18, Anti-IL15, BIRB-796, SCIO-469, VX-702, AMG-548, VX-740, Roflumilast, IC-485, CDC-801, and Mesopram. Combinations include methotrexate or leflunomide and in moderate or severe rheumatoid arthritis cases, cyclosporine.

Nonlimiting additional agents which can also be used in combination with a binding protein to treat rheumatoid arthritis include, but are not limited to, the following: non-steroidal anti-inflammatory drug(s) (NSAIDs); cytokine suppressive anti-inflammatory drug(s) (CSAIDs); CDP-571/BAY-10-3356 (humanized anti-TNFα antibody; Celltech/Bayer); cA2/infliximab (chimeric anti-TNFα antibody; Centocor); 75 kdTNFR-IgG/etanercept (75 kD TNF receptor-IgG fusion protein; Immunex; see *e.g.*, *Arthritis & Rheumatism* (1994) Vol. 37, S295; *J. Invest. Med.* (1996) Vol. 44, 235A); 55 kdTNF-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche); IDEC-CE9.1/SB 210396 (non-depleting primatized anti-CD4 antibody; IDEC/SmithKline; see *e.g.*, *Arthritis & Rheumatism* (1995) Vol. 38, S185); DAB 486-IL-2 and/or DAB 389-IL-2 (IL-2 fusion proteins; Seragen; see *e.g.*, *Arthritis & Rheumatism* (1993) Vol. 36, 1223); Anti-Tac (humanized anti-IL-2Rα; Protein Design Labs/Roche); IL-4 (anti-inflammatory cytokine; DNAX/Schering); IL-10 (SCH 52000; recombinant IL-10, anti-inflammatory cytokine; DNAX/Schering); IL-4; IL-10 and/or IL-4 agonists (*e.g.*, agonist antibodies); IL-1RA (IL-1 receptor antagonist; Synergen/Amgen); anakinra (Kineret®/Amgen); TNF-bp/s-TNF (soluble TNF binding protein; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S284; *Amer. J. Physiol. - Heart and Circulatory Physiology* (1995) Vol. 268, pp. 37-42); R973401 (phosphodiesterase Type IV inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); MK-966 (COX-2 Inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S81); Iloprost (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S82); methotrexate; thalidomide (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282) and thalidomide-related drugs (*e.g.*, Celgen); leflunomide (anti-inflammatory and cytokine inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S131; *Inflammation Research* (1996) Vol. 45, pp. 103-107); tranexamic acid (inhibitor of plasminogen activation; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S284); T-614 (cytokine inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); prostaglandin E1 (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); Tenidap (non-steroidal anti-inflammatory drug; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S280); Naproxen (non-steroidal anti-

inflammatory drug; see *e.g.*, *Neuro Report* (1996) Vol. 7, pp. 1209-1213); Meloxicam (non-steroidal anti-inflammatory drug); Ibuprofen (non-steroidal anti-inflammatory drug); Piroxicam (non-steroidal anti-inflammatory drug); Diclofenac (non-steroidal anti-inflammatory drug); Indomethacin (non-steroidal anti-inflammatory drug); Sulfasalazine (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S281); Azathioprine (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S281); ICE inhibitor (inhibitor of the enzyme interleukin-1β converting enzyme); zap-70 and/or lck inhibitor (inhibitor of the tyrosine kinase zap-70 or lck); VEGF inhibitor and/or VEGF-R inhibitor (inhibitors of vascular endothelial cell growth factor or vascular endothelial cell growth factor receptor; inhibitors of angiogenesis); corticosteroid anti-inflammatory drugs (*e.g.*, SB203580); TNF-convertase inhibitors; anti-IL-12 antibodies; anti-IL-18 antibodies; interleukin-11 (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S296); interleukin-13 (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S308); interleukin -17 inhibitors (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S120); gold; penicillamine; chloroquine; chlorambucil; hydroxychloroquine; cyclosporine; cyclophosphamide; total lymphoid irradiation; anti-thymocyte globulin; anti-CD4 antibodies; CD5-toxins; orally-administered peptides and collagen; lobenzarit disodium; Cytokine Regulating Agents (CRAs) HP228 and HP466 (Houghten Pharmaceuticals, Inc.); ICAM-1 antisense phosphorothioate oligo-deoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); prednisone; orgotein; glycosaminoglycan polysulphate; minocycline; anti-IL2R antibodies; marine and botanical lipids (fish and plant seed fatty acids; see *e.g.*, DeLuca *et al.* (1995) *Rheum. Dis. Clin. North Am.* 21:759-777); auranofin; phenylbutazone; meclofenamic acid; flufenamic acid; intravenous immune globulin; zileuton; azaribine; mycophenolic acid (RS-61443); tacrolimus (FK-506); sirolimus (rapamycin); amiprilose (therafectin); cladribine (2-chlorodeoxyadenosine); methotrexate; bcl-2 inhibitors (see Bruncko, Milan et al., Journal of Medicinal Chemistry (2007), 50(4), 641-662); antivirals and immune modulating agents.

In one embodiment, the binding protein or antigen-binding portion thereof, is administered in combination with one of the following agents for the treatment of rheumatoid arthritis: small molecule inhibitor of KDR, small molecule inhibitor of Tie-2; methotrexate; prednisone; celecoxib; folic acid; hydroxychloroquine sulfate; rofecoxib; etanercept; infliximab; leflunomide; naproxen; valdecoxib; sulfasalazine; methylprednisolone; ibuprofen; meloxicam; methylprednisolone acetate; gold sodium thiomalate; aspirin; azathioprine; triamcinolone acetonide; propxyphene napsylate/apap; folate; nabumetone; diclofenac; piroxicam; etodolac; diclofenac sodium; oxaprozin; oxycodone hcl; hydrocodone bitartrate/apap; diclofenac sodium/misoprostol; fentanyl; anakinra, human recombinant; tramadol hcl; salsalate; sulindac; cyanocobalamin/fa/pyridoxine; acetaminophen; alendronate sodium; prednisolone; morphine

sulfate; lidocaine hydrochloride; indomethacin; glucosamine sulfate/chondroitin; cyclosporine; amitriptyline hcl; sulfadiazine; oxycodone hcl/acetaminophen; olopatadine hcl; misoprostol; naproxen sodium; omeprazole; mycophenolate mofetil; cyclophosphamide; rituximab; IL-1 TRAP; MRA; CTLA4-IG; IL-18 BP; IL-12/23; anti-IL 18; anti-IL 15; BIRB-796; SCIO-469; VX-702; AMG-548; VX-740; Roflumilast; IC-485; CDC-801; and mesopram.

Non-limiting examples of therapeutic agents for inflammatory bowel disease with which a binding protein of the invention can be combined include the following: budenoside; epidermal growth factor; corticosteroids; cyclosporin, sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxygenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1β mAbs; anti-IL-6 mAbs; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-17, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands. The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNFα or IL-1 (e.g.,IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TNFα converting enzyme inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g.,soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R) and antiinflammatory cytokines (e.g.,IL-4, IL-10, IL-11, IL-13 and TGFβ) and bcl-2 inhibitors.

Examples of therapeutic agents for Crohn's disease in which a binding protein can be combined include the following: TNF antagonists, for example, anti-TNF antibodies, ADALIMUMAB (PCT Publication No. WO 97/29131; HUMIRA), CA2 (REMICADE), CDP 571, TNFR-Ig constructs, (p75TNFRIgG (ENBREL) and p55TNFRIgG (LENERCEPT)) inhibitors and PDE4 inhibitors. Antibodies of the invention, or antigen binding portions thereof, can be combined with corticosteroids, for example, budenoside and dexamethasone. Binding proteins of the invention or antigen binding portions thereof, may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid and olsalazine, and agents which interfere with synthesis or action of proinflammatory cytokines such as IL-1, for example, IL-1β converting enzyme inhibitors and IL-1ra. Antibodies of the invention or antigen binding portion thereof may

also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors 6-mercaptopurines. Binding proteins of the invention, or antigen binding portions thereof, can be combined with IL-11. Binding proteins of the invention, or antigen binding portions thereof, can be combined with mesalamine, prednisone, azathioprine, mercaptopurine, infliximab, methylprednisolone sodium succinate, diphenoxylate/atrop sulfate, loperamide hydrochloride, methotrexate, omeprazole, folate, ciprofloxacin/dextrose-water, hydrocodone bitartrate/apap, tetracycline hydrochloride, fluocinonide, metronidazole, thimerosal/boric acid, cholestyramine/sucrose, ciprofloxacin hydrochloride, hyoscyamine sulfate, meperidine hydrochloride, midazolam hydrochloride, oxycodone hcl/acetaminophen, promethazine hydrochloride, sodium phosphate, sulfamethoxazole/trimethoprim, celecoxib, polycarbophil, propoxyphene napsylate, hydrocortisone, multivitamins, balsalazide disodium, codeine phosphate/apap, colesevelam hcl, cyanocobalamin, folic acid, levofloxacin, methylprednisolone, natalizumab and interferon-gamma

Non-limiting examples of therapeutic agents for multiple sclerosis with which binding proteins of the invention can be combined include the following: corticosteroids; prednisolone; methylprednisolone; azathioprine; cyclophosphamide; cyclosporine; methotrexate; 4-aminopyridine; tizanidine; interferon-β1a (AVONEX; Biogen); interferon-β1b (BETASERON; Chiron/Berlex); interferon α-n3) (Interferon Sciences/Fujimoto), interferon-α (Alfa Wassermann/J&J), interferon β1A-IF (Serono/Inhale Therapeutics), Peginterferon α 2b (Enzon/Schering-Plough), Copolymer 1 (Cop-1; COPAXONE; Teva Pharmaceutical Industries, Inc.); hyperbaric oxygen; intravenous immunoglobulin; clabribine; antibodies to or antagonists of other human cytokines or growth factors and their receptors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-23, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Binding proteins of the invention can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD19, CD20, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands. Binding proteins of the invention, may also be combined with agents, such as methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adensosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNFα or IL-1 (e.g.,IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TACE inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g.,soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R), antiinflammatory cytokines (e.g.,IL-4, IL-10, IL-13 and TGFβ) and bcl-2 inhibitors.

Examples of therapeutic agents for multiple sclerosis in which binding proteins of the invention can be combined tinclude interferon-β, for example, IFNβ1a and IFNβ1b; copaxone, corticosteroids, caspase inhibitors, for example inhibitors of caspase-1, IL-1 inhibitors, TNF inhibitors, and antibodies to CD40 ligand and CD80.

The binding proteins of the invention, may also be combined with agents, such as alemtuzumab, dronabinol, Unimed, daclizumab, mitoxantrone, xaliproden hydrochloride, fampridine, glatiramer acetate, natalizumab, sinnabidol, a-immunokine NNSO3, ABR-215062, AnergiX.MS, chemokine receptor antagonists, BBR-2778, calagualine, CPI-1189, LEM (liposome encapsulated mitoxantrone), THC.CBD (cannabinoid agonist) MBP-8298, mesopram (PDE4 inhibitor), MNA-715, anti-IL-6 receptor antibody, neurovax, pirfenidone allotrap 1258 (RDP-1258), sTNF-R1, talampanel, teriflunomide,TGF-beta2, tiplimotide, VLA-4 antagonists (for example, TR-14035, VLA4 Ultrahaler, Antegran-ELAN/Biogen), interferon gamma antagonists, IL-4 agonists.

Non-limiting examples of therapeutic agents for Angina with which binding proteins of the invention can be combined include the following: aspirin, nitroglycerin, isosorbide mononitrate, metoprolol succinate, atenolol, metoprolol tartrate, amlodipine besylate, diltiazem hydrochloride, isosorbide dinitrate, clopidogrel bisulfate, nifedipine, atorvastatin calcium, potassium chloride, furosemide, simvastatin, verapamil hcl, digoxin, propranolol hydrochloride, carvedilol, lisinopril, spironolactone, hydrochlorothiazide, enalapril maleate, nadolol, ramipril, enoxaparin sodium, heparin sodium, valsartan, sotalol hydrochloride, fenofibrate, ezetimibe, bumetanide, losartan potassium, lisinopril/hydrochlorothiazide, felodipine, captopril, bisoprolol fumarate.

Non-limiting examples of therapeutic agents for Ankylosing Spondylitis with which binding proteins of the invention can be combined include the following: ibuprofen, diclofenac and misoprostol, naproxen, meloxicam, indomethacin, diclofenac, celecoxib, rofecoxib, Sulfasalazine, Methotrexate, azathioprine, minocyclin, prednisone, etanercept, infliximab.

Non-limiting examples of therapeutic agents for Asthma with which binding proteins of the invention can be combined include the following: albuterol, salmeterol/fluticasone, montelukast sodium, fluticasone propionate, budesonide, prednisone, salmeterol xinafoate, levalbuterol hcl, albuterol sulfate/ipratropium, prednisolone sodium phosphate, triamcinolone acetonide, beclomethasone dipropionate, ipratropium bromide, azithromycin, pirbuterol acetate, prednisolone, theophylline anhydrous, methylprednisolone sodium succinate, clarithromycin, zafirlukast, formoterol fumarate, influenza virus vaccine, methylprednisolone, amoxicillin trihydrate, flunisolide, allergy injection, cromolyn sodium, fexofenadine hydrochloride, flunisolide/menthol, amoxicillin/clavulanate, levofloxacin, inhaler assist device, guaifenesin,

dexamethasone sodium phosphate, moxifloxacin hcl, doxycycline hyclate, guaifenesin/d-methorphan, p-ephedrine/cod/chlorphenir, gatifloxacin, cetirizine hydrochloride, mometasone furoate, salmeterol xinafoate, benzonatate, cephalexin, pe/hydrocodone/chlorphenir, cetirizine hcl/pseudoephed, phenylephrine/cod/promethazine, codeine/promethazine, cefprozil, dexamethasone, guaifenesin/pseudoephedrine, chlorpheniramine/hydrocodone, nedocromil sodium, terbutaline sulfate, epinephrine, methylprednisolone, metaproterenol sulfate.

Non-limiting examples of therapeutic agents for COPD with which binding proteins of the invention can be combined include the following: albuterol sulfate/ipratropium, ipratropium bromide, salmeterol/fluticasone, albuterol, salmeterol xinafoate, fluticasone propionate, prednisone, theophylline anhydrous, methylprednisolone sodium succinate, montelukast sodium, budesonide, formoterol fumarate, triamcinolone acetonide, levofloxacin, guaifenesin, azithromycin, beclomethasone dipropionate, levalbuterol hcl, flunisolide, ceftriaxone sodium, amoxicillin trihydrate, gatifloxacin, zafirlukast, amoxicillin/clavulanate, flunisolide/menthol, chlorpheniramine/hydrocodone, metaproterenol sulfate, methylprednisolone, mometasone furoate, p-ephedrine/cod/chlorphenir, pirbuterol acetate, p-ephedrine/loratadine, terbutaline sulfate, tiotropium bromide, (R,R)-formoterol, TgAAT, Cilomilast, Roflumilast.

Non-limiting examples of therapeutic agents for HCV with which binding proteins of the invention can be combined include the following: Interferon-alpha-2a, Interferon-alpha-2b, Interferon-alpha con1, Interferon-alpha-n1, Pegylated interferon-alpha-2a, Pegylated interferon-alpha-2b, ribavirin, Peginterferon alfa-2b + ribavirin, Ursodeoxycholic Acid, Glycyrrhizic Acid, Thymalfasin, Maxamine, VX-497 and any compounds that are used to treat HCV through intervention with the following targets: HCV polymerase, HCV protease, HCV helicase, HCV IRES (internal ribosome entry site).

Non-limiting examples of therapeutic agents for Idiopathic Pulmonary Fibrosis with which binding proteins of the invention can be combined include the following: prednisone, azathioprine, albuterol, colchicine, albuterol sulfate, digoxin, gamma interferon, methylprednisolone sod succ, lorazepam, furosemide, lisinopril, nitroglycerin, spironolactone, cyclophosphamide, ipratropium bromide, actinomycin d, alteplase, fluticasone propionate, levofloxacin, metaproterenol sulfate, morphine sulfate, oxycodone hcl, potassium chloride, triamcinolone acetonide, tacrolimus anhydrous, calcium, interferon-alpha, methotrexate, mycophenolate mofetil, Interferon-gamma-1β.

Non-limiting examples of therapeutic agents for Myocardial Infarction with which binding proteins of the invention can be combined include the following: aspirin, nitroglycerin, metoprolol tartrate, enoxaparin sodium, heparin sodium, clopidogrel bisulfate, carvedilol, atenolol, morphine sulfate, metoprolol succinate, warfarin sodium, lisinopril, isosorbide

mononitrate, digoxin, furosemide, simvastatin, ramipril, tenecteplase, enalapril maleate, torsemide, retavase, losartan potassium, quinapril hcl/mag carb, bumetanide, alteplase, enalaprilat, amiodarone hydrochloride, tirofiban hcl m-hydrate, diltiazem hydrochloride, captopril, irbesartan, valsartan, propranolol hydrochloride, fosinopril sodium, lidocaine hydrochloride, eptifibatide, cefazolin sodium, atropine sulfate, aminocaproic acid, spironolactone, interferon, sotalol hydrochloride, potassium chloride, docusate sodium, dobutamine hcl, alprazolam, pravastatin sodium, atorvastatin calcium, midazolam hydrochloride, meperidine hydrochloride, isosorbide dinitrate, epinephrine, dopamine hydrochloride, bivalirudin, rosuvastatin, ezetimibe/simvastatin, avasimibe, cariporide.

Non-limiting examples of therapeutic agents for Psoriasis with which binding proteins of the invention can be combined include the following: small molecule inhibitor of KDR, small molecule inhibitor of Tie-2, calcipotriene, clobetasol propionate, triamcinolone acetonide, halobetasol propionate, tazarotene, methotrexate, fluocinonide, betamethasone diprop augmented, fluocinolone acetonide, acitretin, tar shampoo, betamethasone valerate, mometasone furoate, ketoconazole, pramoxine/fluocinolone, hydrocortisone valerate, flurandrenolide, urea, betamethasone, clobetasol propionate/emoll, fluticasone propionate, azithromycin, hydrocortisone, moisturizing formula, folic acid, desonide, pimecrolimus, coal tar, diflorasone diacetate, etanercept folate, lactic acid, methoxsalen, hc/bismuth subgal/znox/resor, methylprednisolone acetate, prednisone, sunscreen, halcinonide, salicylic acid, anthralin, clocortolone pivalate, coal extract, coal tar/salicylic acid, coal tar/salicylic acid/sulfur, desoximetasone, diazepam, emollient, fluocinonide/emollient, mineral oil/castor oil/na lact, mineral oil/peanut oil, petroleum/isopropyl myristate, psoralen, salicylic acid, soap/tribromsalan, thimerosal/boric acid, celecoxib, infliximab, cyclosporine, alefacept, efalizumab, tacrolimus, pimecrolimus, PUVA, UVB, sulfasalazine.

Non-limiting examples of therapeutic agents for Psoriatic Arthritis with which binding proteins of the invention can be combined include the following: methotrexate, etanercept, rofecoxib, celecoxib, folic acid, sulfasalazine, naproxen, leflunomide, methylprednisolone acetate, indomethacin, hydroxychloroquine sulfate, prednisone, sulindac, betamethasone diprop augmented, infliximab, methotrexate, folate, triamcinolone acetonide, diclofenac, dimethylsulfoxide, piroxicam, diclofenac sodium, ketoprofen, meloxicam, methylprednisolone, nabumetone, tolmetin sodium, calcipotriene, cyclosporine, diclofenac sodium/misoprostol, fluocinonide, glucosamine sulfate, gold sodium thiomalate, hydrocodone bitartrate/apap, ibuprofen, risedronate sodium, sulfadiazine, thioguanine, valdecoxib, alefacept, efalizumab and bcl-2 inhibitors.

Non-limiting examples of therapeutic agents for Restenosis with which binding proteins of the invention can be combined include the following: sirolimus, paclitaxel, everolimus, tacrolimus, Zotarolimus, acetaminophen.

Non-limiting examples of therapeutic agents for Sciatica with which binding proteins of the invention can be combined include the following: hydrocodone bitartrate/apap, rofecoxib, cyclobenzaprine hcl, methylprednisolone, naproxen, ibuprofen, oxycodone hcl/acetaminophen, celecoxib, valdecoxib, methylprednisolone acetate, prednisone, codeine phosphate/apap, tramadol hcl/acetaminophen, metaxalone, meloxicam, methocarbamol, lidocaine hydrochloride, diclofenac sodium, gabapentin, dexamethasone, carisoprodol, ketorolac tromethamine, indomethacin, acetaminophen, diazepam, nabumetone, oxycodone hcl, tizanidine hcl, diclofenac sodium/misoprostol, propoxyphene napsylate/apap, asa/oxycod/oxycodone ter, ibuprofen/hydrocodone bit, tramadol hcl, etodolac, propoxyphene hcl, amitriptyline hcl, carisoprodol/codeine phos/asa, morphine sulfate, multivitamins, naproxen sodium, orphenadrine citrate, temazepam.

Examples of therapeutic agents for SLE (Lupus) in which binding proteins of the invention can be combined include the following: NSAIDS, for example, diclofenac, naproxen, ibuprofen, piroxicam, indomethacin; COX2 inhibitors, for example, Celecoxib, rofecoxib, valdecoxib; anti-malarials, for example, hydroxychloroquine; Steroids, for example, prednisone, prednisolone, budenoside, dexamethasone; Cytotoxics, for example, azathioprine, cyclophosphamide, mycophenolate mofetil, methotrexate; inhibitors of PDE4 or purine synthesis inhibitor, for example Cellcept. Binding proteins of the invention, may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid, olsalazine, Imuran and agents which interfere with synthesis, production or action of proinflammatory cytokines such as IL-1, for example, caspase inhibitors like IL-1β converting enzyme inhibitors and IL-1ra. Binding proteins of the invention may also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors; or molecules that target T cell activation molecules, for example, CTLA-4-IgG or anti-B7 family antibodies, anti-PD-1 family antibodies. Binding proteins of the invention, can be combined with IL-11 or anti-cytokine antibodies, for example, fonotolizumab (anti-IFNg antibody), or anti-receptor receptor antibodies, for example, anti-IL-6 receptor antibody and antibodies to B-cell surface molecules. Antibodies of the invention or antigen binding portion thereof may also be used with LJP 394 (abetimus), agents that deplete or inactivate B-cells, for example, Rituximab (anti-CD20 antibody), lymphostat-B (anti-BlyS antibody), TNF antagonists, for example, anti-TNF antibodies, Adalimumab (PCT Publication No. WO 97/29131; HUMIRA), CA2 (REMICADE), CDP 571, TNFR-Ig constructs, (p75TNFRIgG (ENBREL) and p55TNFRIgG (LENERCEPT)) and bcl-2 inhibitors, because bcl-2 overexpression in transgenic mice has been demonstrated to cause a lupus like phenotype (see Marquina, Regina et al., Journal

of Immunology (2004), 172(11), 7177-7185), therefore inhibition is expected to have therapeutic effects.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of a binding protein of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the binding protein may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the binding protein to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody, or antibody portion, are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of a binding protein of the invention is 0.1-20 mg/kg, for example, 1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the invention described herein are obvious and may be made using suitable equivalents without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

Examples

Example 1: Design, Construction, and Analysis of a DVD-Ig

Example 1.1: Assays Used to Identify and Characterize Parent Antibodies and DVD-Ig

The following assays are used throughout the Examples to identify and characterize parent antibodies and DVD-Ig unless otherwise stated.

Example 1.1.1: Assays Used To Determine Binding and Affinity of Parent Antibodies and DVD-Ig for Their Target Antigen(s)

Example 1.1.1.A: ELISA

Enzyme Linked Immunosorbent Assays to screen for antibodies that bind a desired target antigen are performed as follows. ELISA plates (Corning Costar, Acton, MA) are coated with 50μL/well of 5μg/ml goat anti-mouse IgG Fc specific (Pierce # 31170, Rockford, IL.) in Phosphate Buffered Saline (PBS) overnight at 4°C. Plates are washed once with PBS containing 0.05% Tween-20. Plates are blocked by addition of 200 μL/well blocking solution diluted to 2% in PBS (BioRad #170-6404, Hercules, CA.) for 1 hour at room temperature. Plates are washed once after blocking with PBS containing 0.05% Tween-20.

Fifty microliters per well of, e.g., mouse sera, hybridoma supernatants, or antibody or DVD-Ig preparations diluted in PBS containing 0.1% Bovine Serum Albumin (BSA) (Sigma, St. Louis, MO.) is added to the ELISA plate prepared as described above and incubated for 1 hour at room temperature. Wells are washed three times with PBS containing 0.05% Tween-20. Fifty microliters of biotinylated recombinant purified target antigen diluted to 100ng/mL in PBS containing 0.1% BSA is added to each well and incubated for 1 hour at room temperature. Plates are washed 3 times with PBS containing 0.05% Tween-20. Streptavidin *HRP* (Pierce # 21126, Rockland, IL.) is diluted 1:20000 in PBS containing 0.1% BSA; 50 μL/well is added and the plates incubated for 1 hour at room temperature. Plates are washed 3 times with PBS containing 0.05% Tween-20. Fifty microliters of TMB solution (Sigma # T0440, St. Louis, MO.) is added to each well and incubated for 10 minutes at room temperature. The reaction is stopped by addition of 1N sulphuric acid. Plates are read spectrophotmetrically at a wavelength of 450 nm. Results are shown in Table 3.

Table 3: Direct Bind ELISA Of 104 DVD Constructs With EGFR (seq. 2) Combined With Other Sequences With Various Orientations And Linker Lengths

DVD-Ig ID	N-Term. VD Sequence ID	N-Term. VD EC50 (nM)	N-Term. Ref. Ab. ID	N-Term. Ref. Ab.EC 50 (nM)	HC linker	LC linker	C-Term. VD Sequence ID	C-Term. VD EC50 (nM)	C-Term. Ref. Ab. ID	C-Term. Ref. Ab.EC 50 (nM)
DVD321	EGFR (seq. 2)	6.83	AB064	3.63	S	S	EGFR (seq. 1)	6.83	AB033	1.8
DVD322	EGFR (seq. 1)	1.59	AB033	1.67	S	S	EGFR (seq. 2)	1.59	AB064	3.63
DVD325	EGFR (seq. 2)	5.96	AB064	6.86	S	S	RON	24.99	AB005	0.19
DVD326	RON	0.18	AB005	0.24	S	S	EGFR (seq. 2)	29.93	AB064	8.83
DVD327	EGFR (seq. 2)	3.98	AB064	3.45	S	S	ErbB3 (seq. 1)	485.26	AB062	1.19
DVD328	ErbB3 (seq. 1)	1.14	AB062	1.34	S	S	EGFR (seq. 2)	94.89	AB064	3.83
DVD329	EGFR (seq. 2)	3.82	AB064	4.37	S	S	ErbB3 (seq. 2)	481.28	AB063	0.86
DVD330	ErbB3 (seq. 2)	1.29	AB063	1.11	S	S	EGFR (seq. 2)	224.44	AB064	4.17
DVD331	EGFR (seq. 2)	3.68	AB064	3.89	S	S	CD3	N/A	AB002	N/A
DVD332	CD3	N/A	AB002	N/A	S	S	EGFR (seq. 2)	62.09	AB064	3.95
DVD333	EGFR (seq. 2)	3.55	AB064	4.46	S	S	IGF1R	78.58	AB011	0.24
DVD334	IGF1R	0.19	AB011	0.26	S	S	EGFR (seq. 2)	137.12	AB064	4.28
DVD335	EGFR (seq. 2)	3.77	AB064	5.03	S	S	HGF	159.04	AB012	0.15
DVD336	HGF	0.15	AB012	0.19	S	S	EGFR (seq. 2)	332.05	AB064	5.40
DVD337	EGFR (seq. 2)	6.5	AB064	6.73	S	S	VEGF (seq. 1)	0.57	AB014	0.25
DVD338	VEGF (seq. 1)	0.24	AB014	0.26	S	S	EGFR (seq. 2)	205.54	AB064	7.84
DVD339	EGFR (seq. 2)	5.1	AB064	3.66	S	S	DLL-4	4.01	AB015	0.45
DVD340	DLL-4	0.32	AB015	0.40	S	S	EGFR (seq. 2)	189.14	AB064	3.8
DVD341	EGFR (seq. 2)	3.41	AB064	4.39	S	S	PLGF	0.74	AB047	0.21
DVD342	PLGF	0.2	AB047	0.25	S	S	EGFR (seq. 2)	119.88	AB064	3.77
DVD755	EGFR (seq. 2)	3.26	AB064	4.78	S	S	ErbB3 (seq. 3)	27.68	AB067	1.40
DVD756	ErbB3 (seq. 3)	3.94	AB067	2.03	S	S	EGFR (seq. 2)	48.87	AB064	3.48
DVD757	EGFR (seq. 2)	14.51	AB064	10.69	S	S	VEGF (seq. 2)	528.26	AB070	5.12
DVD758	VEGF (seq. 2)	5.37	AB070	5.91	S	S	EGFR (seq. 2)	561.59	AB064	9.11
DVD759	EGFR (seq. 2)	7.01	AB064	10.25	S	S	VEGF (seq. 3)	127.91	AB071	1.68
DVD760	VEGF (seq. 3)	0.77	AB071	1.57	S	S	EGFR (seq. 2)	328.03	AB064	12.93
DVD765	EGFR (seq. 2)	4.48	AB064	3.63	L	L	EGFR (seq. 1)	4.48	AB033	1.67
DVD766	EGFR (seq. 1)	3.12	AB033	1.8	L	L	EGFR (seq. 2)	3.12	AB064	3.63
DVD767	EGFR (seq. 2)	5.97	AB064	6.86	L	L	RON	0.64	AB005	0.19
DVD768	RON	0.18	AB005	0.24	L	L	EGFR (seq. 2)	20.67	AB064	8.83
DVD769	EGFR (seq. 2)	3.59	AB064	3.45	L	L	ErbB3 (seq. 1)	8.23	AB062	1.19
DVD770	ErbB3 (seq. 1)	1.55	AB062	1.36	L	L	EGFR (seq. 2)	15.24	AB064	6.86
DVD771	EGFR (seq. 2)	4.43	AB064	4.37	L	L	ErbB3 (seq. 2)	1.38	AB063	0.86
DVD772	ErbB3 (seq. 2)	31.42	AB063	1.11	L	L	EGFR (seq. 2)	77.19	AB064	4.17

DVD-Ig ID	N-Term. VD Sequence ID	N-Term. VD EC50 (nM)	N-Term. Ref. Ab. ID	N-Term. Ref. Ab.EC 50 (nM)	HC linker	LC linker	C-Term. VD Sequence ID	C-Term. VD EC50 (nM)	C-Term. Ref. Ab. ID	C-Term. Ref. Ab.EC 50 (nM)
DVD773	EGFR (seq. 2)	4.96	AB064	3.89	L	L	CD3	N/A	AB002	N/A
DVD774	CD3	N/A	AB002	N/A	L	L	EGFR (seq. 2)	19.29	AB064	3.95
DVD775	EGFR (seq. 2)	3.59	AB064	4.46	L	L	IGF1R	2.77	AB011	0.24
DVD776	IGF1R	0.83	AB011	0.26	L	L	EGFR (seq. 2)	286.41	AB064	4.28
DVD777	EGFR (seq. 2)	5.13	AB064	5.03	L	L	HGF	0.96	AB012	0.15
DVD778	HGF	0.17	AB012	0.19	L	L	EGFR (seq. 2)	50.3	AB064	5.40
DVD779	EGFR (seq. 2)	10.57	AB064	6.73	L	L	VEGF (seq. 1)	0.24	AB014	0.25
DVD780	VEGF (seq. 1)	2.19	AB014	0.26	L	L	EGFR (seq. 2)	408.7	AB064	7.84
DVD781	EGFR (seq. 2)	4.19	AB064	3.66	L	L	DLL-4	0.42	AB015	0.45
DVD782	DLL-4	0.37	AB015	0.40	L	L	EGFR (seq. 2)	55.24	AB064	3.8
DVD783	EGFR (seq. 2)	3.88	AB064	4.39	L	L	PLGF	0.36	AB047	0.21
DVD784	PLGF	0.26	AB047	0.25	L	L	EGFR (seq. 2)	41.36	AB064	3.77
DVD787	EGFR (seq. 2)	3.78	AB064	4.78	L	L	ErbB3 (seq. 3)	14.38	AB067	1.40
DVD788	ErbB3 (seq. 3)	3.14	AB067	2.03	L	L	EGFR (seq. 2)	18.04	AB064	3.48
DVD789	EGFR (seq. 2)	14.61	AB064	10.69	L	L	VEGF (seq. 2)	45.95	AB070	5.12
DVD790	VEGF (seq. 2)	15.91	AB070	5.91	L	L	EGFR (seq. 2)	76.76	AB064	9.11
DVD791	EGFR (seq. 2)	8.24	AB064	10.25	L	L	VEGF (seq. 3)	11.62	AB071	1.68
DVD792	VEGF (seq. 3)	0.82	AB071	1.57	L	L	EGFR (seq. 2)	48.61	AB064	12.93
DVD795	EGFR (seq. 2)	6.21	AB064	3.63	L	S	EGFR (seq. 1)	6.21	AB033	1.67
DVD796	EGFR (seq. 1)	1.94	AB033	1.8	L	S	EGFR (seq. 2)	1.94	AB064	3.63
DVD797	EGFR (seq. 2)	4.56	AB064	6.86	L	S	RON	1.16	AB005	0.19
DVD798	RON	0.18	AB005	0.24	L	S	EGFR (seq. 2)	17.94	AB064	8.83
DVD799	EGFR (seq. 2)	5.76	AB064	3.45	L	S	ErbB3 (seq. 1)	20.04	AB062	1.19
DVD800	ErbB3 (seq. 1)	2.06	AB062	1.34	L	S	EGFR (seq. 2)	67.26	AB064	3.83
DVD801	EGFR (seq. 2)	3.91	AB064	4.37	L	S	ErbB3 (seq. 2)	3.28	AB063	0.86
DVD802	ErbB3 (seq. 2)	11.13	AB063	1.11	L	S	EGFR (seq. 2)	726.39	AB064	4.17
DVD803	EGFR (seq. 2)	0.23	AB064	3.89	L	S	CD3	N/A	AB002	N/A
DVD804	CD3	N/A	AB002	N/A	L	S	EGFR (seq. 2)	157.62	AB064	3.95
DVD805	EGFR (seq. 2)	0.48	AB064	4.46	L	S	IGF1R	0.99	AB011	0.24
DVD806	IGF1R	0.4	AB011	0.26	L	S	EGFR (seq. 2)	53.79	AB064	4.28
DVD807	EGFR (seq. 2)	4.85	AB064	5.03	L	S	HGF	9.24	AB012	0.15
DVD808	HGF	0.14	AB012	0.19	L	S	EGFR (seq. 2)	96.36	AB064	5.40
DVD809	EGFR (seq. 2)	7.34	AB064	6.73	L	S	VEGF (seq. 1)	0.23	AB014	0.25
DVD810	VEGF (seq. 1)	0.33	AB014	0.26	L	S	EGFR (seq. 2)	227.99	AB064	7.84
DVD811	EGFR (seq. 2)	3.45	AB064	3.66	L	S	DLL-4	0.99	AB015	0.45
DVD812	DLL-4	0.39	AB015	0.40	L	S	EGFR (seq. 2)	77.26	AB064	3.8
DVD813	EGFR (seq. 2)	3.41	AB064	4.39	L	S	PLGF	0.44	AB047	0.21

DVD-Ig ID	N-Term. VD Sequence ID	N-Term. VD EC50 (nM)	N-Term. Ref. Ab. ID	N-Term. Ref. Ab.EC 50 (nM)	HC linker	LC linker	C-Term. VD Sequence ID	C-Term. VD EC50 (nM)	C-Term. Ref. Ab. ID	C-Term. Ref. Ab.EC 50 (nM)
DVD814	PLGF	0.23	AB047	0.25	L	S	EGFR (seq. 2)	50.43	AB064	3.77
DVD817	EGFR (seq. 2)	5.33	AB064	4.78	L	S	ErbB3 (seq. 3)	18.82	AB067	1.40
DVD818	ErbB3 (seq. 3)	3.32	AB067	2.03	L	S	EGFR (seq. 2)	19.32	AB064	3.48
DVD819	EGFR (seq. 2)	12.2	AB064	10.69	L	S	VEGF (seq. 2)	36.35	AB070	5.12
DVD820	VEGF (seq. 2)	4.08	AB070	5.91	L	S	EGFR (seq. 2)	106.81	AB064	9.11
DVD821	EGFR (seq. 2)	6.96	AB064	10.25	L	S	VEGF (seq. 3)	11.09	AB071	1.68
DVD822	VEGF (seq. 3)	0.72	AB071	1.57	L	S	EGFR (seq. 2)	90.03	AB064	12.93
DVD825	EGFR (seq. 2)	1.67	AB064	3.63	S	L	EGFR (seq. 1)	1.67	AB033	1.67
DVD826	EGFR (seq. 1)	2.27	AB033	1.8	S	L	EGFR (seq. 2)	2.27	AB064	3.63
DVD827	EGFR (seq. 2)	6.28	AB064	6.86	S	L	RON	6.57	AB005	0.19
DVD828	RON	0.2	AB005	0.24	S	L	EGFR (seq. 2)	19.83	AB064	8.83
DVD829	EGFR (seq. 2)	3.81	AB064	3.45	S	L	ErbB3 (seq. 1)	41.4	AB062	1.19
DVD830	ErbB3 (seq. 1)	1.43	AB062	1.34	S	L	EGFR (seq. 2)	42.71	AB064	3.83
DVD831	EGFR (seq. 2)	4.6	AB064	4.37	S	L	ErbB3 (seq. 2)	1.42	AB063	0.86
DVD832	ErbB3 (seq. 2)	1.19	AB063	1.11	S	L	EGFR (seq. 2)	62.39	AB064	4.17
DVD833	EGFR (seq. 2)	3.03	AB064	3.89	S	L	CD3	N/A	AB002	N/A
DVD834	CD3	N/A	AB002	N/A	S	L	EGFR (seq. 2)	26.7	AB064	3.95
DVD835	EGFR (seq. 2)	2.42	AB064	4.46	S	L	IGF1R	3.47	AB011	0.24
DVD836	IGF1R	0.51	AB011	0.26	S	L	EGFR (seq. 2)	56.83	AB064	4.28
DVD837	EGFR (seq. 2)	4.51	AB064	5.03	S	L	HGF	1.29	AB012	0.15
DVD838	HGF	0.19	AB012	0.19	S	L	EGFR (seq. 2)	57.66	AB064	5.40
DVD839	EGFR (seq. 2)	12.53	AB064	6.73	S	L	VEGF (seq. 1)	0.26	AB014	0.25
DVD840	VEGF (seq. 1)	0.18	AB014	0.26	S	L	EGFR (seq. 2)	69.77	AB064	7.84
DVD841	EGFR (seq. 2)	4.06	AB064	3.66	S	L	DLL-4	0.55	AB015	0.45
DVD842	DLL-4	0.36	AB015	0.40	S	L	EGFR (seq. 2)	51.68	AB064	3.8
DVD843	EGFR (seq. 2)	3.65	AB064	4.39	S	L	PLGF	0.37	AB047	0.21
DVD844	PLGF	0.23	AB047	0.25	S	L	EGFR (seq. 2)	45.51	AB064	3.77
DVD847	EGFR (seq. 2)	2.92	AB064	4.78	S	L	ErbB3 (seq. 3)	12.93	AB067	1.40
DVD848	ErbB3 (seq. 3)	3.23	AB067	2.03	S	L	EGFR (seq. 2)	16.74	AB064	3.48
DVD849	EGFR (seq. 2)	12.33	AB064	10.69	S	L	VEGF (seq. 2)	59.58	AB070	5.12
DVD850	VEGF (seq. 2)	10.17	AB070	5.91	S	L	EGFR (seq. 2)	71.83	AB064	9.11
DVD851	EGFR (seq. 2)	9.36	AB064	10.25	S	L	VEGF (seq. 3)	28.44	AB071	1.68
DVD852	VEGF (seq. 3)	0.78	AB071	1.57	S	L	EGFR (seq. 2)	68.11	AB064	12.93

Binding of all DVD-Ig constructs was maintained and comparable to parent antibodies. All N-terminal variable domains bound with a similar high affinity as the parent antibody as well

as the C-terminal variable domains of DVD-Ig constructs DVD322, DVD337, DVD341, DVD765, DVD766, DVD767, DVD771, DVD777, DVD779, DVD781, DVD783, DVD796, DVD803, DVD809, DVD811, DVD813, DVD825, DVD826, DVD831, DVD839, DVD841, and DVD843.

Example 1.1.1.B: Affinity Determination using BIACORE technology

The BIACORE assay (Biacore, Inc, Piscataway, NJ) determines the affinity of antibodies or DVD-Ig with kinetic measurements of on-rate and off-rate constants. Binding of antibodies or DVD-Ig to a target antigen (for example, a purified recombinant target antigen) is determined by surface plasmon resonance-based measurements with a Biacore® 3000 instrument (Biacore® AB, Uppsala, Sweden) using running HBS-EP (10 mM HEPES [pH 7.4], 150 mM NaCl, 3 mM EDTA, and 0.005% surfactant P20) at 25° C. All chemicals are obtained from Biacore® AB (Uppsala, Sweden) or otherwise from a different source as described in the text. For example, approximately 5000 RU of goat anti-mouse IgG, (Fcγ), fragment specific polyclonal antibody (Pierce Biotechnology Inc, Rockford, IL) diluted in 10 mM sodium acetate (pH 4.5) is directly immobilized across a CM5 research grade biosensor chip using a standard amine coupling kit according to manufacturer's instructions and procedures at 25 µg/ml. Unreacted moieties on the biosensor surface are blocked with ethanolamine. Modified carboxymethyl dextran surface in flowcell 2 and 4 is used as a reaction surface. Unmodified carboxymethyl dextran without goat anti-mouse IgG in flow cell 1 and 3 is used as the reference surface. For kinetic analysis, rate equations derived from the 1:1 Langmuir binding model are fitted simultaneously to association and dissociation phases of all eight injections (using global fit analysis) with the use of Biaevaluation 4.0.1 software. Purified antibodies or DVD-Ig are diluted in HEPES-buffered saline for capture across goat anti-mouse IgG specific reaction surfaces. Antibodies to be captured as a ligand (25 µg/ml) are injected over reaction matrices at a flow rate of 5 µl/min. The association and dissociation rate constants, k_{on} ($M^{-1}s^{-1}$) and k_{off} (s^{-1}) are determined under a continuous flow rate of 25 µl/min. Rate constants are derived by making kinetic binding measurements at ten different antigen concentrations ranging from 10 – 200 nM. The equilibrium dissociation constant (M) of the reaction between antibodies or DVD-Igs and the target antigen is then calculated from the kinetic rate constants by the following formula: $K_D = k_{off}/k_{on}$. Binding is recorded as a function of time and kinetic rate constants are calculated. In this assay, on-rates as fast as $10^6 M^{-1}s^{-1}$ and off-rates as slow as $10^{-6} s^{-1}$ can be measured.

Table 4: BIACORE Analysis of Parental Antibodies and DVD Constructs

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	HC Linker	LC Linker	Antigen	k_{on} (M-1s-1)	k_{off} (s-1)	k_D (M)
AB033	EGFR (seq. 1)				FL-hEGFR	2.47E+05	1.13E-03	4.60E-09
AB064	EGFR (seq. 2)				FL-hEGFR	ND	ND	ND
DVD321	EGFR (seq. 2)	EGFR (seq. 1)	Short	Short	FL-hEGFR	5.46E+04	7.39E-04	1.40E-08
DVD322	EGFR (seq. 1)	EGFR (seq. 2)	Short	Short	FL-hEGFR	2.52E+05	1.04E-03	4.10E-09
DVD795	EGFR (seq. 2)	EGFR (seq. 1)	Long	Short	FL-hEGFR	1.29E+04	2.59E-04	2.00E-08
DVD796	EGFR (seq. 1)	EGFR (seq. 2)	Long	Short	FL-hEGFR	3.31E+05	1.21E-03	3.70E-09
DVD765	EGFR (seq. 2)	EGFR (seq. 1)	Long	Long	FL-hEGFR	3.07E+04	2.61E-04	8.50E-09
DVD766	EGFR (seq. 1)	EGFR (seq. 2)	Long	Long	FL-hEGFR	3.06E+05	1.21E-03	3.90E-09
DVD825	EGFR (seq. 2)	EGFR (seq. 1)	Short	Long	FL-hEGFR	1.16E+04	2.95E-04	2.60E-08
DVD826	EGFR (seq. 1)	EGFR (seq. 2)	Short	Long	FL-hEGFR	2.65E+05	1.10E-03	4.20E-09
AB033	EGFR (seq. 1)				d2-7 trunc	3.85E+05	1.06E-03	2.80E-09
AB064	EGFR (seq. 2)				d2-7 trunc	3.70E+04	6.82E-04	1.80E-08
DVD321	EGFR (seq. 2)	EGFR (seq. 1)	Short	Short	d2-7 trunc	5.32E+04	4.55E-04	8.50E-09
DVD322	EGFR (seq. 1)	EGFR (seq. 2)	Short	Short	d2-7 trunc	1.69E+05	5.70E-04	3.40E-09
DVD795	EGFR (seq. 2)	EGFR (seq. 1)	Long	Short	d2-7 trunc	5.65E+04	2.53E-04	4.50E-09
DVD796	EGFR (seq. 1)	EGFR (seq. 2)	Long	Short	d2-7 trunc	2.60E+05	5.02E-04	1.90E-09
DVD765	EGFR (seq. 2)	EGFR (seq. 1)	Long	Long	d2-7 trunc	3.99E+04	2.16E-04	5.40E-09
DVD766	EGFR (seq. 1)	EGFR (seq. 2)	Long	Long	d2-7 trunc	2.06E+05	4.56E-04	2.20E-09
DVD825	EGFR (seq. 2)	EGFR (seq. 1)	Short	Long	d2-7 trunc	3.31E+04	2.40E-04	7.30E-09
DVD826	EGFR (seq. 1)	EGFR (seq. 2)	Short	Long	d2-7 trunc	2.05E+05	4.84E-04	2.40E-09
AB033	EGFR (seq. 1)				d-2-7 Cet	ND	ND	ND
AB064	EGFR (seq. 2)				d-2-7 Cet	2.03E+04	6.60E-04	3.30E-08
DVD321	EGFR (seq. 2)	EGFR (seq. 1)	Short	Short	d-2-7 Cet	2.49E+04	5.56E-04	2.20E-08
DVD322	EGFR (seq. 1)	EGFR (seq. 2)	Short	Short	d-2-7 Cet	1.04E+04	4.34E-04	4.20E-08
DVD795	EGFR (seq. 2)	EGFR (seq. 1)	Long	Short	d-2-7 Cet	2.42E+04	5.52E-04	2.30E-08
DVD796	EGFR (seq. 1)	EGFR (seq. 2)	Long	Short	d-2-7 Cet	1.43E+04	4.48E-04	3.10E-08
DVD765	EGFR (seq. 2)	EGFR (seq. 1)	Long	Long	d-2-7 Cet	2.65E+04	5.76E-04	2.20E-08
DVD766	EGFR (seq. 1)	EGFR (seq. 2)	Long	Long	d-2-7 Cet	1.08E+04	4.45E-04	4.10E-08
DVD825	EGFR (seq. 2)	EGFR (seq. 1)	Short	Long	d-2-7 Cet	2.56E+04	5.50E-04	2.20E-08
DVD826	EGFR (seq. 1)	EGFR (seq. 2)	Short	Long	d-2-7 Cet	1.18E+04	4.45E-04	3.80E-08

Binding of all DVD-Ig constructs characterized by Biacore technology was maintained and comparable to that of parent antibodies. All N-terminal variable domains bound with a similar high affinity as the parent antibody.

Example 1.1.2: Assays Used To Determine theFunctional Activity Of Parent Antibodies And DVD-Ig

Example 1.1.2.A: A431 Cell Binding

Log phase A431 cells were harvested according to standard methods. Each sample containing $5X10^4$ cells (300 μL) was incubated with serial dilution of DVD-Igs in the cold room for 1 hour. Cells were then stained with PE-conjugated goat-anti-human antibody (Jackson ImmunoResearch Cat# 109-115-098) (300 μL) and incubated in the cold room for 30 minutes. The stained cells were analyzed on FACSCalibur HTS (Becton Dickinson, San Jose). The data were analyzed with Prism (GraphPad Software, La Jolla) The data are shown in Table 5

Example 1.1.2.B: Cell Binding Competition Assay

5 nM FITC-conjugated EGFR (seq. 2) were incubated with serial dilutions of DVD-Igs on ice for 15 minutes and then incubated with $5X10^4$ harvested log phase U87MG-de2-7 cells (300 μL) in the cold room for 1 hour. The stained cells were analyzed on FACSCalibur HTS (Becton Dickinson, San Jose). The data were analyzed with Prism (GraphPad Software, La Jolla). The data are shown in Table 5.

Example 1.1.2.C: Western Blot: Total EGFR (Receptor Down-Regulation) And pTyr

Log phase A431 or U87MG-de2-7 cells were plated into 6-well plates at $1X10^6$ cells per well (2 mL) Cells were serum starved the next day for 24 hours. Cells were then treated with 100nM of various antibodies (30 μl) for 1 hour and then stimulated with 15 nM EGF (0.5 μL) for 10 minutes. Cells were then harvested and lyzed on ice with RIPA buffer (150 μL per well) (Sigma). Cell lysates were separated on a SDS-PAGE gel according to standard methods and then transferred to PVDF membrane (Invitrogen). The proteins were detected by Western blotting using various antibody probes. The data are shown in Table 5.

Example 1.1.2.D: Cell Proliferation (Clonogenic) Assay

Log phase A431 cells were plated into 96-well plates at 300 cells per well in 100 μL. Cells were treated with serial dilution of antibodies the next day and incubated for 7-10 days. Cells were fixed with 3.7% paraformaldehyde for 20 minutes and then stained with 0.1% crystal violet for 1 hour. The stained crystal violet was extracted by 10% acetic acid for 1 hour and then quantitated at OD590. The data are shown in Table 5.

Table 5: Screening of EGFR DVD-Igs

DVD ID	Sequence ID	Pos.	HC Linker	LC Linker	Other DVD Domain	A431 Bind.	EGFR Comp.	Ptyr Sign. Inhib.	EGFR Rec. Down Reg.	Cell Prolif. Inhib.
DVD322	EGFR (seq. 2)	C-term	Short	Short	EGFR (seq. 1)	+	-	+	-	ND
DVD321	EGFR (seq. 2)	N-term	Short	Short	EGFR (seq. 1)	-	+	-	+	ND
DVD766	EGFR (seq. 2)	C-term	Long	Long	EGFR (seq. 1)	+	+	+	-	ND
DVD765	EGFR (seq. 2)	N-term	Long	Long	EGFR (seq. 1)	+	+	+	+	+
DVD796	EGFR (seq. 2)	C-term	Long	Short	EGFR (seq. 1)	+	+	-	-	ND
DVD795	EGFR (seq. 2)	N-term	Long	Short	EGFR (seq. 1)	+	+	+	+	+
DVD826	EGFR (seq. 2)	C-term	Short	Long	EGFR (seq. 1)	+	+	-	-	ND
DVD825	EGFR (seq. 2)	N-term	Short	Long	EGFR (seq. 1)	+	+	-	-	ND

Example 1.1.2.E: Down Regulation Of Total EGFR In Multiple Human Cancer Cell Lines By MSD Assay

Log phase cells (A431, A431NS, A549 or HN5) were plated into 96-well plate at $1X10^4$ cells/well (100 μL) The next day, the cells were treated with different antibodies (30 μL) at 100nM for 2 hours at 37°C. The cells were then harvested and total EGFR levels were quantitated with Whole Cell Lysate Kit-Total EGFR Assay (Meso Scale Discovery Cat# K151CKD-2) according to the manufacturer's protocol. The data are shown in Table 6.

Table 6: Down regulation of total EGFR in multiple human cancer cell lines by MSD Assay

Percentage of Control (100nM)				
Cell Lines	EGFR (seq. 2)	EGFR (seq. 1)	DVD795	EGFR (seq. 2) + EGFR (seq. 1)
A431	97	30	14	32
A431NS	98	44	24	43
A549	91	29	7	29
HN5	102	53	11	36
NCI-H1975	94	35	15	25
OVCAR5	94	34	12	36
PC3	108	57	20	72
U87MG	96	58	18	59
U87MG-EGFRwt	86	57	13	39
U87MG-de2-7	90	49	21	52

DVD795 showed down regulation of total EGFR in all human cancer cell lines tested.

Example 1.1.2.G: Cytokine Bioassay

The ability of an anti-cytokine parent antibody or DVD-Ig containing anti-cytokine sequences to inhibit or neutralize a target cytokine bioactivity is analyzed by determinating inhibitory potential of the antibody or DVD-Ig. For example, the ability of an anti-IL-4 antibody to inhibit IL-4 mediated IgE production may be used. For example, human naive B cells are isolated from peripheral blood, respectively, buffy coats by Ficoll-paque density centrifugation, followed by magnetic separation with MACS beads (Miltenyi Biotech) specific for human sIgD FITC labeled goat F(ab)2 antibodies followed by anti-FITC MACS beads. Magnetically sorted naive B cells are adjusted to 3 x 10^5 cells per ml in XV15 and plated out in 100 µl per well of 96-well plates in a 6 x 6 array in the center of the plate, surrounded by PBS filled wells during the 10 days of culture at 37° C in the presence of 5% CO_2. One plate each is prepared per antibody to be tested, consisting of 3 wells each of un-induced and induced controls and quintuplicate repeats of antibody titrations starting at 7 µg/ml and running in 3-fold dilution down to 29 ng/ml final concentrations added in 50µl four times concentrated pre-dilution. To induce IgE production, rhIL-4 at 20 ng/ml plus anti-CD40 monoclonal antibody (Novartis) at 0.5 µg/ml final concentrations in 50 µl each are added to each well, and IgE concentrations are determined at the end of the culture period by a standard sandwich ELISA method.

Example 1.1.2.H: Cytokine Release Assay

The ability of a parent antibody or DVD-Ig to cause cytokine release is analyzed. Peripheral blood is withdrawn from three healthy donors by venipuncture into heparized vacutainer tubes. Whole blood is diluted 1:5 with RPMI-1640 medium and placed in 24-well tissue culture plates at 0.5 mL per well. The anti-cytokine antibodies (e.g., anti-IL-4) are diluted into RPMI-1640 and placed in the plates at 0.5 mL/well to give final concentrations of 200, 100, 50, 10, and 1 µg/mL. The final dilution of whole blood in the culture plates is 1:10. LPS and PHA are added to separate wells at 2µg/mL and 5µg/mL final concentration as a positive control for cytokine release. Polyclonal human IgG is used as negative control antibody. The experiment is performed in duplicate. Plates are incubated at 37°C at 5% CO_2. Twenty-four hours later the contents of the wells are transferred into test tubes and spun for 5 minutes at 1200 rpm. Cell-free supernatants are collected and frozen for cytokine assays. Cells left over on the plates and in the tubes are lysed with 0.5 mL of lysis solution, and placed at –20°C and thawed. 0.5 mL of medium is added (to bring the volume to the same level as the cell-free supernatant samples) and the cell preparations are collected and frozen for cytokine assays. Cell-free supernatants and cell lysates are assayed for cytokine levels by ELISA, for example, for levels of IL-8, IL-6, IL-1β, IL-1RA, TNF-α.

Example 1.1.2.I: Redirected Cytotoxicity (rCTL) Assay: FACS based

Human CD3+ T cells were isolated from previously frozen isolated PBMC by a negative selection enrichment column (R&D Cat.#HTCC-525). T cells were stimulated for 4 days in flasks coated with 10μg/mL anti-CD3 (OKT-3, BD) and 2μg/mL anti-CD28 (CD28.2, Abcam) in complete RPMI media (L-glutamine, 55mM β-ME, Pen/Strep, 10%FCS). T cells were rested overnight in 30U/mL IL-2 (Peprotech) before using in assay. DoHH2 or Raji target cells were labeled with PKH26 (Sigma) according to manufacturer's instructions. RPMI 1640 media (no phenol, Invitrogen) containing L-glutamine and 10% FBS (Hyclone) was used throughout the rCTL assay.

Effector T cells (E) and targets (T) were plated at 10^5 and 10^4 cells/well in 96-well plates (Costar #3799), respectively to give an E:T ratio of 10:1. DVD-Ig molecules were appropriately diluted to obtain concentration-dependent titration curves. After an overnight incubation cells were pelleted and washed with PBS once before resuspending in 100 μL PBS containing 0.1%BSA (Invitrogen) and 0.5μg/mL propidium iodide (BD). FACS data was collected on a FACSCanto machine (Becton Dickinson, San Jose)and analyzed in Flowjo (Treestar). The percent live targets in the DVD-Ig treated samples divided by the percent total targets (control, no treatment) was calculated to determine percent specific lysis. The data is graphed and IC50s are calculated in Prism (Graphpad Software, La Jolla).

Example 1.1.Redirected Cytotoxicity (rCTL) Assay: Impedence based

T cells were prepared as above. EGFR-expressing target cells were allowed to adhere to ACEA RT-CES 96-well plates (ACEA Bio, San Diego) overnight. Effector T cells (E) and targets (T) were then plated at $2X10^5$ and $2X10^4$ cells/well to give an E:T ratio of 10:1. DVD-Ig molecules were appropriately diluted to obtain concentration-dependent titration curves. The cell indexes of targets in the DVD-Ig treated samples were divided by the cell indexes of control targets (no treatment) to calculate percent specific lysis. The data was graphed and IC50s were calculated in Prism (Graphpad Software, La Jolla). The data is shown in Table 7

Table 7: rCTL Activity of EGFR (seq. 2) Containing DVD-Igs

DVD ID	Sequence ID	Position	HC Linker	LC Linker	Other DVD Domain	rCTL activity IC50 (pM)
DVD774	EGFR (seq. 2)	C-term	Long	Long	CD3	0.2
DVD773	EGFR (seq. 2)	N-term	Long	Long	CD3	16
DVD804	EGFR (seq. 2)	C-term	Long	Short	CD3	0.09
DVD803	EGFR (seq. 2)	N-term	Long	Short	CD3	42
DVD332	EGFR (seq. 2)	C-term	Short	Short	CD3	0.03
DVD331	EGFR (seq. 2)	N-term	Short	Short	CD3	>200 nM
DVD834	EGFR (seq. 2)	C-term	Short	Long	CD3	0.08
DVD833	EGFR (seq. 2)	N-term	Short	Long	CD3	12

All DVD-Igs containing VDs from AB002 in either the N-terminal or C-terminal position showed killing in the rCTL assay.

Example 1.1.2.C: Cytokine Cross-Reactivity Study

The ability of an anti-cytokine parent antibody or DVD-Ig directed to a cytokine(s) of interest to cross react with other cytokines is analyzed. Parent antibodies or DVD-Ig are immobilized on a BIAcore biosensor matrix. An anti-human Fc mAb is covalently linked via free amine groups to the dextran matrix by first activating carboxyl groups on the matrix with 100mM N-hydroxysuccinimide (NHS) and 400mM N-Ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC). Approximately 50μL of each antibody or DVD-Ig preparation at a concentration of 25μg/mL, diluted in sodium acetate, pH4.5, is injected across the activated biosensor and free amines on the protein are bound directly to the activated carboxyl groups. Typically, 5000 Resonance Units (RU's) are immobilized. Unreacted matrix EDC-esters are deactivated by an injection of 1 M ethanolamine. A second flow cell is prepared as a reference standard by immobilizing human IgG1/K using the standard amine coupling kit. SPR measurements are performed using the CM biosensor chip. All antigens to be analyzed on the biosensor surface are diluted in HBS-EP running buffer containing 0.01% P20.

To examine the cytokine binding specificity, excess cytokine of interest (100nM, e.g., soluble recombinant human) is injected across the anti-cytokine parent antibody or DVD-Ig immobilized biosensor surface (5 minute contact time). Before injection of the cytokine of interest and immediately afterward, HBS-EP buffer alone flows through each flow cell. The net difference in the signals between the baseline and the point corresponding to approximately 30 seconds after completion of cytokine injection are taken to represent the final binding value. Again, the response is measured in Resonance Units. Biosensor matrices are regenerated using 10mM HCl before injection of the next sample where a binding event is observed, otherwise running buffer was injected over the matrices. Human cytokines (e.g., IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-22, IL-23, IL-27, TNF-α, TNF-β, and IFN-γ, for example) are also simultaneously injected over the immobilized mouse IgG1/K reference surface to record any nonspecific binding background. By preparing a reference and reaction surface, Biacore can automatically subtract the reference surface data from the reaction surface data in order to eliminate the majority of the refractive index change and injection noise. Thus, it is possible to ascertain the true binding response attributed to an anti-cytokine antibody or DVD-Ig binding reaction.

When a cytokine of interest is injected across immobilized anti-cytokine antibody, significant binding is observed. 10mM HCl regeneration completely removes all non-covalently

associated proteins. Examination of the sensorgram shows that immobilized anti-cytokine antibody or DVD-Ig binding to soluble cytokine is strong and robust. After confirming the expected result with the cytokine of interest, the panel of remaining recombinant human cytokines is tested, for each antibody or DVD-Ig separately. The amount of anti-cytokine antibody or DVD-Ig bound or unbound cytokine for each injection cycle is recorded. The results from three independent experiments are used to determine the specificity profile of each antibody or DVD-Ig. Antibodies or DVD-Ig with the expected binding to the cytokine of interest and no binding to any other cytokine are selected.

Example 1.1.2.D: Tissue Cross Reactivity

Tissue cross reactivity studies are done in three stages, with the first stage including cryosections of 32 tissues, second stage inluding up to 38 tissues, and the 3rd stage including additional tissues from 3 unrelated adults as described below. Studies are done typically at two dose levels.

Stage 1: Cryosections (about 5 μm) of human tissues (32 tissues (typically: Adrenal Gland, Gastrointestinal Tract, Prostate, Bladder, Heart, Skeletal Muscle, Blood Cells, Kidney, Skin, Bone Marrow, Liver, Spinal Cord, Breast, Lung, Spleen, Cerebellum, Lymph Node, Testes, Cerebral Cortex, Ovary, Thymus, Colon, Pancreas, Thyroid, Endothelium, Parathyroid, Ureter, Eye, Pituitary, Uterus, Fallopian Tube and Placenta) from one human donor obtained at autopsy or biopsy) are fixed and dried on object glass. The peroxidase staining of tissue sections is performed, using the avidin-biotin system.

Stage 2: Cryosections (about 5 μm) of human tissues 38 tissues (including adrenal, blood, blood vessel, bone marrow, cerebellum, cerebrum, cervix, esophagus, eye, heart, kidney, large intestine, liver, lung, lymph node, breast mammary gland, ovary, oviduct, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, small intestine, spinal cord, spleen, stomach, striated muscle, testis, thymus, thyroid, tonsil, ureter, urinary bladder, and uterus) from 3 unrelated adults obtained at autopsy or biopsy) are fixed and dried on object glass. The peroxidase staining of tissue sections is performed, using the avidin-biotin system.

Stage 3: Cryosections (about 5 μm) of cynomolgus monkey tissues (38 tissues (including adrenal, blood, blood vessel, bone marrow, cerebellum, cerebrum, cervix, esophagus, eye, heart, kidney, large intestine, liver, lung, lymph node, breast mammary gland, ovary, oviduct, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, small intestine, spinal cord, spleen, stomach, striated muscle, testis, thymus, thyroid, tonsil, ureter, urinary bladder, and uterus) from 3 unrelated adult monkeys obtained at autopsy or biopsy) are fixed and

dried on object glass. The peroxidase staining of tissue sections is performed, using the avidin-biotin system.

The antibody or DVD-Ig is incubated with the secondary biotinylated anti-human IgG and developed into immune complex. The immune complex at the final concentrations of 2 and 10 μg/mL of antibody or DVD-Ig is added onto tissue sections on object glass and then the tissue sections are reacted for 30 minutes with a avidin-biotin-peroxidase kit. Subsequently, DAB (3,3'-diaminobenzidine), a substrate for the peroxidase reaction, is applied for 4 minutes for tissue staining. Antigen-Sepharose beads are used as positive control tissue sections. Target antigen and human serum blocking studies serve as additional controls. The immune complex at the final concentrations of 2 and 10 μg/mL of antibody or DVD-Ig is pre-incubated with target antigen (final concentration of 100 μg/ml) or human serum (final concentration 10%) for 30 minutes, and then added onto the tissue sections on object glass and then the tissue sections are reacted for 30 minutes with a avidin-biotin-peroxidase kit. Subsequently, DAB (3,3'-diaminobenzidine), a substrate for the peroxidase reaction, is applied for 4 minutes for tissue staining.

Any specific staining is judged to be either an expected (e.g., consistent with antigen expression) or unexpected reactivity based upon known expression of the target antigen in question. Any staining judged specific is scored for intensity and frequency. The tissue staining between stage 2 (human tissue) and stage 3 (cynomolgus monkey tissue) is either judged to be similar or different.

Example 1.1.2.E: Tumoricidal Effect Of A Parent or DVD-Ig Antibody *In Vitro*

Parent antibodies or DVD-Ig that bind to target antigens on tumor cells may be analyzed for tumoricidal activity. Briefly, parent antibodies or DVD-Ig are diluted in D-PBS-BSA (Dulbecco's phosphate buffered saline with 0.1%BSA) and added to human tumor cells at final concentrations of 0.01 μg/mL to 100 μg/mL. The plates are incubated at 37 °C in a humidified, 5% CO_2 atmosphere for 3 days. The number of live cells in each well is quantified using MTS reagents according to the manufacturer's instructions (Promega, Madison, WI) to determine the percent of tumor growth inhibition. Wells without antibody treatment are used as controls of 0% inhibition whereas wells without cells are considered to show 100% inhibition.

For assessment of apoptosis, caspase-3 activation is determined by the following protocol: antibody-treated cells in 96 well plates are lysed in 120 μl of 1x lysis buffer (1.67mM Hepes, pH 7.4, 7mM KCl, 0.83mM $MgCl_2$, 0.11mM EDTA, 0.11mM EGTA, 0.57% CHAPS, 1mM DTT, 1x protease inhibitor cocktail tablet; EDTA-free; Roche Pharmaceuticals, Nutley, NJ) at room temperature with shaking for 20 minutes. After cell lysis, 80 μl of a caspase-3 reaction buffer (48mM Hepes, pH 7.5, 252mM sucrose, 0.1% CHAPS, 4mM DTT, and 20 μM Ac-DEVD-AMC substrate; Biomol Research Labs, Inc., Plymouth Meeting, PA) is added and the plates are

incubated for 2 hours at 37^{0}C. The plates are read on a 1420 VICTOR Multilabel Counter (Perkin Elmer Life Sciences, Downers Grove, IL) using the following settings: excitation= 360/40, emission= 460/40. An increase of fluorescence units from antibody-treated cells relative to the isotype antibody control-treated cells is seen, which is indicative of apoptosis.

Example 1.1.2.F: Inhibition Of Receptor Activation By Antibodies or DVD-Ig *In Vitro*

Parent antibodies or DVD-Ig that bind to cell receptors or their ligands may be tested for *inhibition of receptor activation. Parent antibodies or DVD-Ig diluted in* D-PBS-BSA (Dulbecco's phosphate buffered saline with 0.1%BSA) are added to human carcinoma cells at final concentrations of 0.01 μg/mL to 100 μg/mL. The plates are incubated at 37 °C in a humidified, 5% CO_2 atmosphere for 1h. Growth factors (e.g., IGF1 or IGF2) at concentration of 1-100 ng/mL are added to the cells for 5-15 minutes to stimulate receptor (e.g., IGF1R) autophosphorylation. Wells without antibody treatment are used as controls of 0% inhibition whereas wells without growth factor stimulation are considered to show 100% inhibition. Cell lysates are made by incubation with cell extraction buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 1 mM sodium orthovanadate, 1% Triton X-100, 10% Glycerol, 0.1% SDS, and protease inhibitor cocktail). Phospho-IGF1R in these cell lysates is determined using specific ELISA kits purchased from R&D System (Minneapolis, MN).

Example 1.1.2.G: Efficacy Of An Anti-Tumor Cell Antigen Antibody or DVD-Ig By Itself Or In Combination With Chemotherapy On The Growth Of Human Carcinoma Xenografts (Subcutaneous Flank, Orthotopic, Or Spontaneous Metastases)

Human cancer cells are grown *in vitro* to 99% viability, 85% confluence in tissue culture flasks. SCID female or male mice (Charles Rivers Labs) at 19-25 grams are ear tagged and shaved. Mice are then inoculated subcutaneously into the right flank with 0.2 ml of 2 x 10^6 human tumor cells (1:1 matrigel) on study day 0. Administration (IP, Q3D/ week) of vehicle (PBS), antibody or DVD-Ig, and/or chemotherapy is initiated after mice are size matched into separate cages of mice with mean tumor volumes of approximately 150 to 200 mm^3. The tumors are measured by a pair of calipers twice a week starting on approximately day 10 post inoculation and the tumor volumes calculated according to the formula $V = L \times W^2/2$ (V: volume, mm^3; L: length, mm. W: width, mm). Reduction in tumor volume is seen in animals treated with antibody or DVD-Ig alone or in combination with chemotherapy relative to tumors in animals that received only vehicle or an isotype control mAb.

Example 1.1.2.H: Binding of Monoclonal Antibodies to the Surface of Human Tumor Cell Lines as Assessed by Flow Cytometry

Stable cell lines overexpressing cell-surface antigen of interest or human tumor cell lines were harvested from tissue culture flasks and resuspended in phosphate buffered saline (PBS)

containing 5% fetal calf serum (PBS/FCS). Prior to staining, human tumor cells were incubated on ice with human IgG at 200μg/ml in PBS/FCS. 1-5 x10^5 cells were incubated with antibody or DVD-Ig (1-2 μg/mL) in PBS/FCS for 30-60 minutes on ice. Cells were washed twice and 100μl of goat anti mouse IgG- phycoerythrin (1:300 dilution in PBS/BSA) (Jackson ImmunoResearch, West Grove, PA, Cat.#115-115-164) was added. After 30 minutes incubation on ice, cells were washed twice and resuspended in PBS/FCS. Fluorescence was measured using a Becton Dickinson FACSCalibur (Becton Dickinson, San Jose, CA). Tha data are shown in Table 8.

Table 8: FACS binding for EGFR (seq. 2) Containing DVD-Igs

DVD ID	Sequence ID	Position	HC Linker	LC Linker	Other DVD Domain	Jurkat FACS EC50 (pM)	U287IΔ2-7 FACS % of parent
DVD774	EGFR (seq. 2)	C-term	Long	Long	CD3	890	70
DVD773	EGFR (seq. 2)	N-term	Long	Long	CD3	>10 nM	105
DVD804	EGFR (seq. 2)	C-term	Long	Short	CD3	650	70
DVD803	EGFR (seq. 2)	N-term	Long	Short	CD3	>10 nM	110
DVD332	EGFR (seq. 2)	C-term	Short	Short	CD3	775	40
DVD331	EGFR (seq. 2)	N-term	Short	Short	CD3	>50 nM	115
DVD834	EGFR (seq. 2)	C-term	Short	Long	CD3	700	65
DVD833	EGFR (seq. 2)	N-term	Short	Long	CD3	>10 nM	110

All DVD-Igs showed binding to their cell surface targets. The N-terminal domains of DVD-Igs bound their targets on the cell surface as well as or better than the parent antibody. Binding can be restored or improved by adjusting linker length

Example 1.2: Generation Of Parent Monoclonal Antibodies to a Human Antigen of Interest

Parent mouse mAbs able to bind to and neutralize a human antigen of interest and a variant thereof are obtained as follows:

Example 1.2.A: Immunization Of Mice With a Human Antigen of Interest

Twenty micrograms of recombinant purified human antigen (e.g., IGF1,2) mixed with complete Freund's adjuvant or Immunoeasy adjuvant (Qiagen, Valencia, CA) is injected subcutaneously into five 6-8 week-old Balb/C, five C57B/6 mice, and five AJ mice on Day 1. On days 24, 38, and 49, twenty micrograms of recombinant purified human antigen variant mixed with incomplete Freund's adjuvant or Immunoeasy adjuvant is injected subcutaneously into the same mice. On day 84 or day 112 or day 144, mice are injected intravenously with 1 μg recombinant purified human antigen of interest.

Example 1.2.B: Generation of Hybridoma

Splenocytes obtained from the immunized mice described in Example 1.2.A are fused with SP2/O-Ag-14 cells at a ratio of 5:1 according to the established method described in Kohler, G. and Milstein, C. (1975) Nature 256: 495 to generate hybridomas. Fusion products are plated in

selection media containing azaserine and hypoxanthine in 96-well plates at a density of $2.5x10^6$ spleen cells per well. Seven to ten days post fusion, macroscopic hybridoma colonies are observed. Supernatant from each well containing hybridoma colonies is tested by ELISA for the presence of antibody to the antigen of interest (as described in Example 1.2.A). Supernatants displaying antigen-specific activity are then tested for activity (as described in the assays of Example 1.1.2), for example, the ability to neutralize the antigen of interest in a bioassay such as that described in Example 1.1.2.A).

Example 1.2.C: Identification And Characterization Of Parent Monoclonal Antibodies to a Human Target Antigen of Interest

Example 1.2.C.1: Analyzing Parent Monoclonal Antibody Neutralizing Activity

Hybridoma supernatants are assayed for the presence of parent antibodies that bind an antigen of interest, generated according to Examples 1.2.A and 1.2.B, and are also capable of binding a variant of the antigen of interest ("antigen variant"). Supernatants with antibodies positive in both assays are then tested for their antigen neutralization potency, for example, in the cytokine bioassay of Example 1.1.2.A. The hybridomas producing antibodies with IC_{50} values in the bioassay less than 1000pM, in an embodiment, less than 100pM are scaled up and cloned by limiting dilution. Hybridoma cells are expanded into media containing 10% low IgG fetal bovine serum (Hyclone #SH30151, Logan, UT.). On average, 250 mL of each hybridoma supernatant (derived from a clonal population) is harvested, concentrated and purified by protein A affinity chromatography, as described in Harlow, E. and Lane, D. 1988 "Antibodies: A Laboratory Manual". The ability of purified mAbs to inhibit the activity of its target antigen is determined, for example, using the cytokine bioassay as described in Example 1.1.2.A.

Example 1.2.C.2: Analyzing Parent Monoclonal Antibody Cross-Reactivity To Cynomolgus Target Antigen Of Interest

To determine whether the selected mAbs described herein recognize cynomolgus antigen of interest, BIACORE analysis is conducted as described herein (Example 1.1.1.B) using recombinant cynomolgus target antigen. In addition, neutralization potencies of mAbs against recombinant cynomolgus antigen of interest may also be measured in the cytokine bioassay (Example 1.1.2.A). MAbs with good cyno cross-reactivity (in an embodiment, within 5-fold of reactivity for human antigen) are selected for future characterization.

Example 1.2.D: Determination Of The Amino Acid Sequence Of The Variable Region For Each Murine Anti-Human Monoclonal Antibody

Isolation of the cDNAs, expression and characterization of the recombinant anti-human mouse mAbs is conducted as follows. For each amino acid sequence determination,

approximately 1 x 10^6 hybridoma cells are isolated by centrifugation and processed to isolate total RNA with Trizol (Gibco BRL/Invitrogen, Carlsbad, CA.) following manufacturer's instructions. Total RNA is subjected to first strand DNA synthesis using the SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA) per the manufacturers instructions. Oligo(dT) is used to prime first-strand synthesis to select for poly(A)+ RNA. The first-strand cDNA product is then amplified by PCR with primers designed for amplification of murine immunoglobulin variable regions (Ig-Primer Sets, Novagen, Madison, WI). PCR products are resolved on an agarose gel, excised, purified, and then subcloned with the TOPO Cloning kit into pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA) and transformed into TOP10 chemically competent E. coli (Invitrogen, Carlsbad, CA). Colony PCR is performed on the transformants to identify clones containing insert. Plasmid DNA is isolated from clones containing insert using a QIAprep Miniprep kit (Qiagen, Valencia, CA). Inserts in the plasmids are sequenced on both strands to determine the variable heavy or variable light chain DNA sequences using M13 forward and M13 reverse primers (Fermentas Life Sciences, Hanover MD). Variable heavy and variable light chain sequences of the mAbs are identified. In an embodiment, the selection criteria for a panel of lead mAbs for next step development (humanization) includes the following:

- The antibody does not contain any N-linked glycosylation sites (NXS), except from the standard one in CH2
- The antibody does not contain any extra cysteines in addition to the normal cysteines in every antibody
- The antibody sequence is aligned with the closest human germline sequences for VH and VL and any unusual amino acids should be checked for occurrence in other natural human antibodies
- N-terminal Glutamine (Q) is changed to Glutamic acid (E) if it does not affect the activity of the antibody. This will reduce heterogeneity due to cyclization of Q
- Efficient signal sequence cleavage is confirmed by Mass Spectrophotometry. This can be done with COS cell or 293 cell material
- The protein sequence is checked for the risk of deamidation of Asn that could result in loss of activity
- The antibody has a low level of aggregation
- The antibody has solubility >5-10 mg/ml (in research phase); >25 mg/ml
- The antibody has a normal size (5-6 nm) by Dynamic Light Scattering (DLS)
- The antibody has a low charge heterogeneity
- The antibody lacks cytokine release (see Example 1.1.2.B)
- The antibody has specificity for the intended cytokine (see Example 1.1.2.C)
- The antibody lacks unexpected tissue cross reactivity (see Example 1.1.2.D)

- The antibody has similarity between human and cynomolgus tissue cross reactivity (see Example 1.1.2.D)

Example 1.2.2: Recombinant Humanized Parent Antibodies

Example 1.2.2.1: Construction And Expression Of Recombinant Chimeric Anti Human Parent Antibodies

The DNA encoding the heavy chain constant region of murine anti-human parent mAbs is replaced by a cDNA fragment encoding the human IgG1 constant region containing 2 hinge-region amino acid mutations by homologous recombination in bacteria. These mutations are a leucine to alanine change at position 234 (EU numbering) and a leucine to alanine change at position 235 (Lund et al. (1991) J. Immunol. 147: 2657). The light chain constant region of each of these antibodies is replaced by a human kappa constant region. Full-length chimeric antibodies are transiently expressed in COS cells by co-transfection of chimeric heavy and light chain cDNAs ligated into the pBOS expression plasmid (Mizushima and Nagata (1990) Nucl. Acids Res. 18: 5322). Cell supernatants containing recombinant chimeric antibody are purified by Protein A Sepharose chromatography and bound antibody is eluted by addition of acid buffer. Antibodies are neutralized and dialyzed into PBS.

The heavy chain cDNA encoding a chimeric mAb is co-transfected with its chimeric light chain cDNA (both ligated in the pBOS vector) into COS cells. Cell supernatant containing recombinant chimeric antibody is purified by Protein A Sepharose chromatography and bound antibody is eluted by addition of acid buffer. Antibodies are neutralized and dialyzed into PBS.

The purified chimeric anti-human parent mAbs are then tested for their ability to bind (by Biacore) and for functional activity, e.g., to inhibit the cytokine induced production of IgE as described in Examples 1.1.1.B and 1.1.2.B. Chimeric mAbs that maintain the activity of the parental hybridoma mAbs are selected for future development.

Example 1.2.2.2: Construction And Expression Of Humanized Anti Human ParentAntibodies

Example 1.2.2.2.A: Selection Of Human Antibody Frameworks

Each murine variable heavy and variable light chain gene sequence is separately aligned against 44 human immunoglobulin germline variable heavy chain or 46 germline variable light chain sequences (derived from NCBI Ig Blast website at *http://www.ncbi.nlm.nih.gov/igblast/retrieveig.html.*) using Vector NTI software.

Humanization is based on amino acid sequence homology, CDR cluster analysis, frequency of use among expressed human antibodies, and available information on the crystal structures of human antibodies. Taking into account possible effects on antibody binding, VH-

VL pairing, and other factors, murine residues are mutated to human residues where murine and human framework residues are different, with a few exceptions. Additional humanization strategies are designed based on an analysis of human germline antibody sequences, or a subgroup thereof, that possessed a high degree of homology, i.e., sequence similarity, to the actual amino acid sequence of the murine antibody variable regions.

Homology modeling is used to identify residues unique to the murine antibody sequences that are predicted to be critical to the structure of the antibody combining site, the CDRs. Homology modeling is a computational method whereby approximate three dimensional coordinates are generated for a protein. The source of initial coordinates and guidance for their further refinement is a second protein, the reference protein, for which the three dimensional coordinates are known and the sequence of which is related to the sequence of the first protein. The relationship among the sequences of the two proteins is used to generate a correspondence between the reference protein and the protein for which coordinates are desired, the target protein. The primary sequences of the reference and target proteins are aligned with coordinates of identical portions of the two proteins transferred directly from the reference protein to the target protein. Coordinates for mismatched portions of the two proteins, e.g., from residue mutations, insertions, or deletions, are constructed from generic structural templates and energy refined to insure consistency with the already transferred model coordinates. This computational protein structure may be further refined or employed directly in modeling studies. The quality of the model structure is determined by the accuracy of the contention that the reference and target proteins are related and the precision with which the sequence alignment is constructed.

For the murine mAbs, a combination of BLAST searching and visual inspection is used to identify suitable reference structures. Sequence identity of 25% between the reference and target amino acid sequences is considered the minimum necessary to attempt a homology modeling exercise. Sequence alignments are constructed manually and model coordinates are generated with the program Jackal (see Petrey, D. et al. (2003) Proteins 53 (Suppl. 6): 430–435).

The primary sequences of the murine and human framework regions of the selected antibodies share significant identity. Residue positions that differ are candidates for inclusion of the murine residue in the humanized sequence in order to retain the observed binding potency of the murine antibody. A list of framework residues that differ between the human and murine sequences is constructed manually.

The likelihood that a given framework residue would impact the binding properties of the antibody depends on its proximity to the CDR residues. Therefore, using the model structures, the residues that differ between the murine and human sequences are ranked according to their distance from any atom in the CDRs. Those residues that fell within 4.5 Å of any CDR atom are

identified as most important and are recommended to be candidates for retention of the murine residue in the humanized antibody (i.e., back mutation).

In silico constructed humanized antibodies are constructed using oligonucleotides. For each variable region cDNA, 6 oligonucleotides of 60-80 nucleotides each are designed to overlap each other by 20 nucleotides at the 5' and/or 3' end of each oligonucleotide. In an annealing reaction, all 6 oligonulceotides are combined, boiled, and annealed in the presence of dNTPs. DNA polymerase I, Large (Klenow) fragment (New England Biolabs #M0210, Beverley, MA.) is added to fill-in the approximately 40bp gaps between the overlapping oligonucleotides. PCR is performed to amplify the entire variable region gene using two outermost primers containing overhanging sequences complementary to the multiple cloning site in a modified pBOS vector (Mizushima, S. and Nagata, S. (1990) Nucleic Acids Res. 18: 17). The PCR products derived from each cDNA assembly are separated on an agarose gel and the band corresponding to the predicted variable region cDNA size is excised and purified. The variable heavy region is inserted in-frame onto a cDNA fragment encoding the human IgG1 constant region containing 2 hinge-region amino acid mutations by homologous recombination in bacteria. These mutations are a leucine to alanine change at position 234 (EU numbering) and a leucine to alanine change at position 235 (Lund et al. (1991) J. Immunol. 147: 2657). The variable light chain region is inserted in-frame with the human kappa constant region by homologous recombination. Bacterial colonies are isolated and plasmid DNA extracted. cDNA inserts are sequenced in their entirety. Correct humanized heavy and light chains corresponding to each antibody are co-transfected into COS cells to transiently produce full-length humanized anti-human antibodies. Cell supernatants containing recombinant chimeric antibody are purified by Protein A Sepharose chromatography and bound antibody is eluted by addition of acid buffer. Antibodies are neutralized and dialyzed into PBS.

Example 1.2.2.3: <u>Characterization Of Humanized Antibodies</u>

The ability of purified humanized antibodies to inhibit a functional activity is determined, e.g., using the cytokine bioassay as described in Examples 1.1.2.A. The binding affinities of the humanized antibodies to recombinant human antigen are determined using surface plasmon resonance (Biacore®) measurement as described in Example 1.1.1.B. The IC_{50} values from the bioassays and the affinity of the humanized antibodies are ranked. The humanized mAbs that fully maintain the activity of the parental hybridoma mAbs are selected as candidates for future development. The top 2-3 most favorable humanized mAbs are further characterized.

Example 1.2.2.3.A: <u>Pharmacokinetic Analysis Of Humanized Antibodies</u>

Pharmacokinetic studies are carried out in Sprague-Dawley rats and cynomolgus monkeys. Male and female rats and cynomolgus monkeys are dosed intravenously or

subcutaneously with a single dose of 4mg/kg mAb and samples are analyzed using antigen capture ELISA, and pharmacokinetic parameters are determined by noncompartmental analysis. Briefly, ELISA plates are coated with goat anti-biotin antibody (5 mg/ml, 4°C, overnight), blocked with Superblock (Pierce), and incubated with biotinylated human antigen at 50 ng/ml in 10% Superblock TTBS at room temperature for 2 hours. Serum samples are serially diluted (0.5% serum, 10% Superblock in TTBS) and incubated on the plate for 30 minutes at room temperature. Detection is carried out with HRP-labeled goat anti human antibody and concentrations are determined with the help of standard curves using the four parameter logistic fit. Values for the pharmacokinetic parameters are determined by non-compartmental model using WinNonlin software (Pharsight Corporation, Mountain View, CA). Humanized mAbs with good pharmacokinetics profile (T1/2 is 8-13 days or better, with low clearance and excellent *bioavailability 50-100%) are selected.*

Example 1.2.2.3.B: Physicochemical And In Vitro Stability Analysis Of Humanized Monoclonal Antibodies

Size exclusion chromatography

Antibodies are diluted to 2.5 mg/mL with water and 20 mL is analyzed on a Shimadzu HPLC system using a TSK gel G3000 SWXL column (Tosoh Bioscience, cat# k5539-05k). Samples are eluted from the column with 211 mM sodium sulfate, 92 mM sodium phosphate, pH 7.0, at a flow rate of 0.3 mL/minutes. The HPLC system operating conditions are the following:

Mobile phase:	211 mM Na_2SO_4, 92 mM $Na_2HPO_4*7H_2O$, pH 7.0
Gradient:	Isocratic
Flow rate:	0.3 mL/minute
Detector wavelength:	280 nm
Autosampler cooler temp:	4°C
Column oven temperature:	Ambient
Run time:	50 minutes

The purity data for the DVD-Igs is shown in Table 9.

Table 9: Purity of Parent Antibodies and DVD-Ig Constructs as Determined by Size Exclusion Chromatography

DVD ID	Sequence ID	Position	HC Linker	LC Linker	Other DVD Domain	% monomer (purity)
DVD774	EGFR (seq. 2)	C-term	Long	Long	CD3	85
DVD773	EGFR (seq. 2)	N-term	Long	Long	CD3	97
DVD804	EGFR (seq. 2)	C-term	Long	Short	CD3	87
DVD803	EGFR (seq. 2)	N-term	Long	Short	CD3	91
DVD332	EGFR (seq. 2)	C-term	Short	Short	CD3	91
DVD331	EGFR (seq. 2)	N-term	Short	Short	CD3	99
DVD834	EGFR (seq. 2)	C-term	Short	Long	CD3	82
DVD833	EGFR (seq. 2)	N-term	Short	Long	CD3	99
DVD322	EGFR (seq. 2)	C-term	Short	Short	EGFR (seq. 1)	97.8
DVD321	EGFR (seq. 2)	N-term	Short	Short	EGFR (seq. 1)	94.4
DVD766	EGFR (seq. 2)	C-term	Long	Long	EGFR (seq. 1)	95.1
DVD765	EGFR (seq. 2)	N-term	Long	Long	EGFR (seq. 1)	92.5
DVD796	EGFR (seq. 2)	C-term	Long	Short	EGFR (seq. 1)	97.9
DVD795	EGFR (seq. 2)	N-term	Long	Short	EGFR (seq. 1)	96.2
DVD826	EGFR (seq. 2)	C-term	Short	Long	EGFR (seq. 1)	95.7
DVD825	EGFR (seq. 2)	N-term	Short	Long	EGFR (seq. 1)	95.0

DVD-Igs showed an excellent SEC profile with most DVD-Ig showing >90% monomer. This DVD-Ig profile is similar to that observed for parent antibodies.

SDS-PAGE

Antibodies are analyzed by sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) under both reducing and non-reducing conditions. Adalimumab lot AFP04C is used as a control. For reducing conditions, the samples are mixed 1:1 with 2X tris glycine SDS-PAGE sample buffer (Invitrogen, cat# LC2676, lot# 1323208) with 100 mM DTT, and heated at 60°C for 30 minutes. For non-reducing conditions, the samples are mixed 1:1 with sample buffer and heated at 100°C for 5 minutes. The reduced samples (10 mg per lane) are loaded on a 12% pre-cast tris-glycine gel (Invitrogen, cat# EC6005box, lot# 6111021), and the non-reduced samples (10 mg per lane) are loaded on an 8%-16% pre-cast tris-glycine gel (Invitrogen, cat# EC6045box, lot# 6111021). SeeBlue Plus 2 (Invitrogen, cat#LC5925, lot# 1351542) is used as a molecular weight marker. The gels are run in a XCell SureLock mini cell gel box (Invitrogen, cat# EI0001) and the proteins are separated by first applying a voltage of 75 to stack the samples in the gel, followed by a constant voltage of 125 until the dye front reached the bottom of the gel. The running buffer used is 1X tris glycine SDS buffer, prepared from a 10X tris glycine SDS buffer (ABC, MPS-79-080106)). The gels are stained overnight with colloidal blue stain (Invitrogen cat# 46-7015, 46-7016) and destained with Milli-Q water until the background is clear. The stained gels are then scanned using an Epson Expression scanner (model 1680, S/N *DASX003641*).

Sedimentation Velocity Analysis

Antibodies are loaded into the sample chamber of each of three standard two-sector carbon epon centerpieces. These centerpieces have a 1.2 cm optical path length and are built with sapphire windows. PBS is used for a reference buffer and each chamber contained 140 μL. All samples are examined simultaneously using a 4-hole (AN-60Ti) rotor in a Beckman ProteomeLab XL-I analytical ultracentrifuge (serial # PL106C01).

Run conditions are programmed and centrifuge control is performed using ProteomeLab (v5.6). The samples and rotor are allowed to thermally equilibrate for one hour prior to analysis (20.0 ± 0.1 °C). Confirmation of proper cell loading is performed at 3000 rpm and a single scan is recorded for each cell. The sedimentation velocity conditions are the following:

Sample Cell Volume: 420 mL
Reference Cell Volume: 420 mL
Temperature: 20°C
Rotor Speed: 35,000 rpm
Time: 8:00 hours
UV Wavelength: 280 nm
Radial Step Size: 0.003 cm
Data Collection: One data point per step without signal averaging.
Total Number of Scans: 100

LC-MS molecular weight measurement of intact antibodies

Molecular weight of intact antibodies are analyzed by LC-MS. Each antibody is diluted to approximately 1 mg/mL with water. An 1100 HPLC (Agilent) system with a protein microtrap (Michrom Bioresources, Inc, cat# 004/25109/03) is used to desalt and introduce 5 mg of the sample into an API Qstar pulsar i mass spectrometer (Applied Biosystems). A short gradient is used to elute the samples. The gradient is run with mobile phase A (0.08% FA, 0.02% TFA in HPLC water) and mobile phase B (0.08% FA and 0.02% TFA in acetonitrile) at a flow rate of 50 mL/minute. The mass spectrometer is operated at 4.5 kvolts spray voltage with a scan range from 2000 to 3500 mass to charge ratio.

LC-MS molecular weight measurement of antibody light and heavy chains

Molecular weight measurement of antibody light chain (LC), heavy chain (HC) and deglycosylated HC are analyzed by LC-MS. Aantibody is diluted to 1 mg/mL with water and the sample is reduced to LC and HC with a final concentration of 10 mM DTT for 30 minutes at 37°C. To deglycosylate the antibody, 100 mg of the antibody is incubated with 2 mL of PNGase F, 5 mL of 10% N-octylglucoside in a total volume of 100 mL overnight at 37 °C. After

deglycosylation the sample is reduced with a final concentration of 10 mM DTT for 30 minutes at 37°C. An Agilent 1100 HPLC system with a C4 column (Vydac, cat# 214TP5115, S/N 060206537204069) is used to desalt and introduce the sample (5 mg) into an API Qstar pulsar i mass spectrometer (Applied Biosystems). A short gradient is used to elute the sample. The gradient is run with mobile phase A (0.08% FA, 0.02% TFA in HPLC water) and mobile phase B (0.08% FA and 0.02% TFA in acetonitrile) at a flow rate of 50 mL/minute. The mass spectrometer is operated at 4.5 kvolts spray voltage with a scan range from 800 to 3500 mass to charge ratio.

Peptide mapping

Antibody is denatured for 15 minutes at room temperature with a final concentration of 6 M guanidine hydrochloride in 75 mM ammonium bicarbonate. The denatured samples are reduced with a final concentration of 10 mM DTT at 37°C for 60 minutes, followed by alkylation with 50 mM iodoacetic acid (IAA) in the dark at 37°C for 30 minutes. Following alkylation, the sample is dialyzed overnight against four liters of 10 mM ammonium bicarbonate at 4°C. The dialyzed sample is diluted to 1 mg/mL with 10 mM ammonium bicarbonate, pH 7.8 and 100 mg of antibody is either digested with trypsin (Promega, cat# V5111) or Lys-C (Roche, cat# 11 047 825 001) at a 1:20 (w/w) trypsin/Lys-C:antibody ratio at 37°C for 4 hrs. Digests are quenched with 1 mL of 1 N HCl. For peptide mapping with mass spectrometer detection, 40 mL of the digests are separated by reverse phase high performance liquid chromatography (RPHPLC) on a C18 column (Vydac, cat# 218TP51, S/N NE9606 10.3.5) with an Agilent 1100 HPLC system. The peptide separation is run with a gradient using mobile phase A (0.02% TFA and 0.08% FA in HPLC grade water) and mobile phase B (0.02% TFA and 0.08% FA in acetonitrile) at a flow rate of 50 mL/minutes. The API QSTAR Pulsar i mass spectromer is operated in positive mode at 4.5 kvolts spray voltage and a scan range from 800 to 2500 mass to charge ratio.

Disulfide Bond Mapping

To denature the antibody, 100 mL of the antibody is mixed with 300 mL of 8 M guanidine HCl in 100 mM ammonium bicarbonate. The pH is checked to ensure that it is between 7 and 8 and the samples are denatured for 15 minutes at room temperature in a final concentration of 6 M guanidine HCl. A portion of the denatured sample (100 mL) is diluted to 600 mL with Milli-Q water to give a final guanidine-HCl concentration of 1 M. The sample (220 mg) is digested with either trypsin (Promega, cat # V5111, lot# 22265901) or Lys-C (Roche, cat# *11047825001, lot# 12808000) at a 1:50 trypsin or 1:50 Lys-C: antibody (w/w) ratios* (4.4 mg enzyme: 220 mg sample) at 37°C for approximately 16 hours. An additional 5 mg of trypsin or Lys-C is added to the samples and digestion is allowed to proceed for an additional 2 hours at 37°C. Digestions are stopped by adding 1 mL of TFA to each sample. Digested samples are

separated by RPHPLC using a C18 column (Vydac, cat# 218TP51 S/N NE020630-4-1A) on an Agilent HPLC system. The separation is run with the same gradient used for peptide mapping using mobile phase A (0.02% TFA and 0.08% FA in HPLC grade water) and mobile phase B (0.02% TFA and 0.08% FA in acetonitrile) at a flow rate of 50 mL/minute. The HPLC operating conditions are the same as those used for peptide mapping. The API QSTAR Pulsar i mass spectromer is operated in positive mode at 4.5 kvolts spray voltage and a scan range from 800 to 2500 mass-to-charge ratio. Disulfide bonds are assigned by matching the observed MWs of peptides with the predicted MWs of tryptic or Lys-C peptides linked by disulfide bonds.

Free sulfhydryl determination

The method used to quantify free cysteines in an antibody is based on the reaction of Ellman's reagent, 5,5¢- dithio-bis (2-nitrobenzoic acid) (DTNB), with sulfhydryl groups (SH) which gives rise to a characteristic chromophoric product, 5-thio-(2-nitrobenzoic acid) (TNB). The reaction is illustrated in the formula:

DTNB + RSH ® RS-TNB + TNB- + H+

The absorbance of the TNB- is measured at 412 nm using a Cary 50 spectrophotometer. An absorbance curve is plotted using dilutions of 2 mercaptoethanol (b-ME) as the free SH standard and the concentrations of the free sulfhydryl groups in the protein are determined from absorbance at 412 nm of the sample.

The b-ME standard stock is prepared by a serial dilution of 14.2 M b-ME with HPLC grade water to a final concentration of 0.142 mM. Then standards in triplicate for each concentration are prepared. Antibody is concentrated to 10 mg/mL using an amicon ultra 10,000 MWCO centrifugal filter (Millipore, cat# UFC801096, lot# L3KN5251) and the buffer is changed to the formulation buffer used for adalimumab (5.57 mM sodium phosphate monobasic, 8.69 mM sodium phosphate dibasic, 106.69 mM NaCl, 1.07 mM sodium citrate, 6.45 mM citric acid, 66.68 mM mannitol, pH 5.2, 0.1% (w/v) Tween). The samples are mixed on a shaker at room temperature for 20 minutes. Then 180 mL of 100 mM Tris buffer, pH 8.1 is added to each sample and standard followed by the addition of 300 mL of 2 mM DTNB in 10 mM phosphate buffer, pH 8.1. After thorough mixing, the samples and standards are measured for absorption at 412 nm on a Cary 50 spectrophotometer. The standard curve is obtained by plotting the amount of free SH and OD_{412} nm of the b-ME standards. Free SH content of samples are calculated based on this curve after subtraction of the blank.

Weak Cation Exchange Chromatography

Antibody is diluted to 1 mg/mL with 10 mM sodium phosphate, pH 6.0. Charge heterogeneity is analyzed using a Shimadzu HPLC system with a WCX-10 ProPac analytical

column (Dionex, cat# 054993, S/N 02722). The samples are loaded on the column in 80% mobile phase A (10 mM sodium phosphate, pH 6.0) and 20% mobile phase B (10 mM sodium phosphate, 500 mM NaCl, pH 6.0) and eluted at a flow rate of 1.0 mL/minute.

Oligosaccharide Profiling

Oligosaccharides released after PNGase F treatment of antibody are derivatized with 2-aminobenzamide (2-AB) labeling reagent. The fluorescent-labeled oligosaccharides are separated by normal phase high performance liquid chromatography (NPHPLC) and the different forms of oligosaccharides are characterized based on retention time comparison with known standards.

The antibody is first digested with PNGaseF to cleave N-linked oligosaccharides from the Fc portion of the heavy chain. The antibody (200 mg) is placed in a 500 mL Eppendorf tube along with 2 mL PNGase F and 3 mL of 10% N-octylglucoside. Phosphate buffered saline is added to bring the final volume to 60 mL. The sample is incubated overnight at 37°C in an Eppendorf thermomixer set at 700 RPM. Adalimumab lot AFP04C is also digested with PNGase F as a control.

After PNGase F treatment, the samples are incubated at 95°C for 5 minutes in an Eppendorf thermomixer set at 750 RPM to precipitate out the proteins, then the samples are placed in an Eppendorf centrifuge for 2 minutes at 10,000 RPM to spin down the precipitated proteins. The supernatent containing the oligosaccharides are transferred to a 500 mL Eppendorf tube and dried in a speed-vac at 65°C.

The oligosaccharides are labeled with 2AB using a 2AB labeling kit purchased from Prozyme (cat# GKK-404, lot# 132026). The labeling reagent is prepared according to the manufacturer's instructions. Acetic acid (150 mL, provided in kit) is added to the DMSO vial (provided in kit) and mixed by pipeting the solution up and down several times. The acetic acid/DMSO mixture (100 mL) is transferred to a vial of 2-AB dye (just prior to use) and mixed until the dye is fully dissolved. The dye solution is then added to a vial of reductant (provided in kit) and mixed well (labeling reagent). The labeling reagent (5 mL) is added to each dried oligosaccharide sample vial, and mixed thoroughly. The reaction vials are placed in an Eppendorf thermomixer set at 65°C and 700-800 RPM for 2 hours of reaction.

After the labeling reaction, the excess fluorescent dye is removed using GlycoClean S Cartridges from Prozyme (cat# GKI-4726). Prior to adding the samples, the cartridges are washed with 1 mL of milli-Q water followed with 5 ishes of 1 mL 30% acetic acid solution. Just prior to adding the samples, 1 mL of acetonitrile (Burdick and Jackson, cat# AH015-4) is added to the cartridges.

After all of the acetonitrile passed through the cartridge, the sample is spotted onto the center of the freshly washed disc and allowed to adsorb onto the disc for 10 minutes. The disc is washed with 1 mL of acetonitrile followed by five ishes of 1 mL of 96% acetonitrile. The cartridges are placed over a 1.5 mL Eppendorf tube and the 2-AB labeled oligosaccharides are eluted with 3 ishes (400 mL each ish) of milli Q water.

The oligosaccharides are separated using a Glycosep N HPLC (cat# GKI-4728) column connected to a Shimadzu HPLC system. The Shimadzu HPLC system consisted of a system controller, degasser, binary pumps, autosampler with a sample cooler, and a fluorescent detector.

Stability at Elevated Temperatures

The buffer of antibody is either 5.57 mM sodium phosphate monobasic, 8.69 mM sodium phosphate dibasic, 106.69 mM NaCl, 1.07 mM sodium citrate, 6.45 mM citric acid, 66.68 mM mannitol, 0.1% (w/v) Tween, pH 5.2; or 10 mM histidine, 10 mM methionine, 4% mannitol, pH 5.9 using Amicon ultra centrifugal filters. The final concentration of the antibodies is adjusted to 2 mg/mL with the appropriate buffers. The antibody solutions are then filter sterized and 0.25 mL aliquots are prepared under sterile conditions. The aliquots are left at either -80°C, 5°C, 25°C, or 40°C for 1, 2 or 3 weeks. At the end of the incubation period, the samples are analyzed by size exclusion chromatography and SDS-PAGE.

The stability samples are analyzed by SDS-PAGE under both reducing and non-reducing conditions. The procedure used is the same as described herein. The gels are stained overnight with colloidal blue stain (Invitrogen cat# 46-7015, 46-7016) and destained with Milli-Q water until the background is clear. The stained gels are then scanned using an Epson Expression scanner (model 1680, S/N DASX003641). To obtain more sensitivity, the same gels are silver stained using silver staining kit (Owl Scientific) and the recommended procedures given by the manufacturer is used.

Example 1.2.2.3.C: Efficacy Of A Humanized Monoclonal Antibody By Itself Or In Combination With Chemotherapy On The Growth Of Human Carcinoma Xenografts

Human cancer cells are grown *in vitro* to 99% viability, 85% confluence in tissue culture flasks. SCID female or male mice (Charles Rivers Labs) at 19-25 grams, are ear tagged and shaved. Mice are then inoculated subcutaneously into the right flank with 0.2 ml of 2 x 10^6 human tumor cells (1:1 matrigel) on study day 0. Administration (IP, Q3D/ week) of vehicle (PBS), humanized antibody, and/or chemotherapy is initiated after mice are size matched into separate cages of mice with mean tumor volumes of approximately 150 to 200 mm^3. The tumors are measured by a pair of calipers twice a week starting on approximately day 10 post inoculation and the tumor volumes calculated according to the formula $V = L \times W^2/2$ (V: volume, mm^3; L: length, mm; W: width, mm). Reduction in tumor volume is seen in animals treated with mAb

alone or in combination with chemotherapy relative to tumors in animals that received only vehicle or an isotype control mAb.

Example 1.4: Generation of a DVD-Ig

DVD-Ig molecules capable of binding two antigens are constructed using two parent monoclonal antibodies, one against human antigen A, and the other against human antigen B, selected as described herein.

Example 1.4.1: Generation of a DVD-Ig having two linker lengths

A constant region containing γ1 Fc with mutations at 234, and 235 to eliminate ADCC/CDC effector functions is used. Four different anti-A/B DVD-Ig constructs are generated: 2 with short linker and 2 with long linker, each in two different domain orientations: V_A-V_B-C and V_B-V_A-C (see Table 8). The linker sequences, derived from the N-terminal sequence of human Cl/Ck or CH1 domain, are as follows:

For DVDAB constructs:

light chain (if anti-A has λ):Short linker: QPKAAP (SEQ ID NO: 15); Long linker: QPKAAPSVTLFPP (SEQ ID NO: 16)

light chain (if anti-A has κ):Short linker: TVAAP (SEQ ID NO: 13); Long linker: TVAAPSVFIFPP (SEQ ID NO: 14)

heavy chain (γ1): Short linker: ASTKGP (SEQ ID NO: 21); Long linker: ASTKGPSVFPLAP (SEQ ID NO: 22)

For DVDBA constructs:

light chain (if anti-B has λ):Short linker: QPKAAP (SEQ ID NO: 15); Long linker: QPKAAPSVTLFPP (SEQ ID NO: 16)

light chain (if anti-B has k):Short linker: TVAAP (SEQ ID NO: 13); Long linker: TVAAPSVFIFPP (SEQ ID NO: 14)

heavy chain (γ1): Short linker: ASTKGP (SEQ ID NO: 21); Long linker: ASTKGPSVFPLAP (SEQ ID NO: 22)

Heavy and light chain constructs are subcloned into the pBOS expression vector, and expressed in COS cells, followed by purification by Protein A chromatography. The purified materials are subjected to SDS-PAGE and SEC analysis.

The Table 10 below describes the heavy chain and light chain constructs used to express each anti-A/B DVD-Ig protein.

Table 10: Constructs to Express Anti-A/B DVD-Ig Proteins

DVD-Ig protein	Heavy chain construct	Light chain construct
DVDABSL	DVDABHC-SL	DVDABLC-SL
DVDABLL	DVDABHC-LL	DVDABLC-LL
DVDBASL	DVDBAHC-SL	DVDBALC-SL
DVDBALL	DVDBAHC-LL	DVDBALC-LL

Example 1.4.2: Molecular cloning of DNA constructs for DVDABSL and DVDABLL

To generate heavy chain constructs DVDABHC-LL and DVDABHC-SL, VH domain of A antibody is PCR amplified using specific primers (3' primers contain short/long liner sequence for SL/LL constructs, respectively); meanwhile VH domain of B antibody is amplified using specific primers (5' primers contains short/long liner sequence for SL/LL constructs, respectively). Both PCR reactions are performed according to standard PCR techniques and procedures. The two PCR products are gel-purified, and used together as overlapping template for the subsequent overlapping PCR reaction. The overlapping PCR products are subcloned into Srf I and Sal I double digested pBOS-hCγ1,z non-a mammalian expression vector (Abbott) by using standard homologous recombination approach.

To generate light chain constructs DVDABLC-LL and DVDABLC-SL, VL domain of A antibody is PCR amplified using specific primers (3' primers contain short/long liner sequence for SL/LL constructs, respectively); meanwhile VL domain of B antibody is amplified using specific primers (5' primers contains short/long liner sequence for SL/LL constructs, respectively). Both PCR reactions are performed according to standard PCR techniques and procedures. The two PCR products are gel-purified, and used together as overlapping template for the subsequent overlapping PCR reaction using standard PCR conditions. The overlapping PCR products are subcloned into Srf I and Not I double digested pBOS-hCk mammalian expression vector (Abbott) by using standard homologous recombination approach. Similar approach has been used to generate DVDBASL and DVDBALL as described below:

Example 1.4.3: Molecular cloning of DNA constructs for DVDBASL and DVDBALL:

To generate heavy chain constructs DVDBAHC-LL and DVDBAHC-SL, VH domain of antibody B is PCR amplified using specific primers (3' primers contain short/long liner sequence for SL/LL constructs, respectively); meanwhile VH domain of antibody A is amplified using specific primers (5' primers contains short/long liner sequence for SL/LL constructs, respectively). Both PCR reactions are performed according to standard PCR techniques and procedures. The two PCR products are gel-purified, and used together as overlapping template for the subsequent overlapping PCR reaction using standard PCR conditions. The overlapping

PCR products are subcloned into Srf I and Sal I double digested pBOS-hCγ1,z non-a mammalian expression vector (Abbott) by using standard homologous recombination approach.

To generate light chain constructs DVDBALC-LL and DVDBALC-SL, VL domain of antibody B is PCR amplified using specific primers (3' primers contain short/long liner sequence for SL/LL constructs, respectively); meanwhile VL domain of antibody A is amplified using specific primers (5' primers contains short/long liner sequence for SL/LL constructs, respectively). Both PCR reactions are performed according to standard PCR techniques and procedures. The two PCR products are gel-purified, and used together as overlapping template for the subsequent overlapping PCR reaction using standard PCR conditions. The overlapping PCR products are subcloned into Srf I and Not I double digested pBOS-hCk mammalian expression vector (Abbott) by using standard homologous recombination approach.

Example 1.4.4: Construction and Expression of Additional DVD-Ig

Example 1.4.4.1: Preparation of DVD-Ig Vector Constructs

Parent antibody amino acid sequences for specific antibodies, which recognize specific antigens or epitopes thereof, for incorporation into a DVD-Ig can be obtained by preparation of hybridomas as described above or can be obtained by sequencing known antibody proteins or nucleic acids. In addition, known sequences can be obtained from the literature. The sequences can be used to synthesize nucleic acids using standard DNA synthesis or amplification technologies and assembling the desired antibody fragments into expression vectors, using standard recombinant DNA technology, for expression in cells.

DVD-Ig sequences are cloned into a pHyb-C vector or a pHyb-E vector (see US Patent Application Serial No. 61/021,282) according to standard methods.

The pHyb-C vector includes an SV40 eukaryotic origin of replication, a cytomegalovirus eukaryotic expression promoter (pCMV), a Tripartite leader sequence (TPL), a splice donor site (SD), an Adenovirus major late enhancer element (enh MLP), a splice acceptor site (SA), an open reading frame (ORF) region for a gene of interest followed by a poly A signal (pA), a dyad symmetry element (DS), an Epstein Barr virus-derived eukaryotic origin of replication (OriP), a repeat region (FR), an ampillicin resistance marker (AmpR) and a bacterial origin of replication (pMB1ori).

The pHyb-E vector includes a SV-40 eukaryotic origin of replication, an EF-1a eukaryotic promoter, an open reading frame (ORF) region for a gene of interest followed by a poly A signal (pA), a dyad symmetry element (DS), an Epstein Barr virus-derived eukaryotic origin of replication (OriP), a repeat region (FR), an ampillicin resistance marker (AmpR) and a

bacterial origin of replication (pMB1ori). Exemplary pHyb-E vectors include the pHybE-hCk, pHybE-hCl, and pHybE-hCg1,z,non-a (see US Patent Application Serial No. 61/021,282).

Example 1.4.4.2: Transfection and expression in 293 cells

The DVD-Ig vector constructs are tranfected into 293 cells for production of DVD-Ig protein. The 293 transient transfection procedure used is a modification of the methods published in Durocher et al. (2002) Nucl. Acids Res. 30(2): E9 and Pham et al. (2005) Biotech. Bioengineering 90(3): 332-44. Reagents that were used in the transfection included:

- HEK 293-6E cells (human embryonic kidney cell line stably expressing EBNA1; obtained from National Research Council Canada) cultured in disposable Erlenmeyer flasks in a humidified incubator set at 130 rpm, 37°C and 5% CO_2.
- Culture medium: FreeStyle 293 Expression Medium (Invitrogen 12338-018) plus 25 µg/mL Geneticin (G418) (Invitrogen 10131-027) and 0.1% Pluronic F-68 (Invitrogen 24040-032).
- Transfection medium: FreeStyle 293 Expression Medium plus 10 mM HEPES (Invitrogen 15630-080).
- Polyethylenimine (PEI) stock: 1 mg/mL sterile stock solution, pH 7.0, prepared with linear 25kDa PEI (Polysciences) and stored at less than -15°C.
- Tryptone Feed Medium: 5% w/v sterile stock of Tryptone N1 (Organotechnie, 19554) in FreeStyle 293 Expression Medium.

Cell preparation for transfection: Approximately 2 - 4 hours prior to transfection, HEK 293-6E cells are harvested by centrifugation and resuspended in culture medium at a cell density of approximately 1 million viable cells per mL. For each transfection, 40 mL of the cell suspension is transferred into a disposable 250-mL Erlenmeyer flask and incubated for 2 - 4 hours.

Transfection: The transfection medium and PEI stock are prewarmed to room temperature (RT). For each transfection, 25µg of plasmid DNA and 50µg of polyethylenimine (PEI) are combined in 5 mL of transfection medium and incubated for 15 – 20 minutes at RT to allow the DNA:PEI complexes to form. For the BR3-Ig transfections, 25µg of BR3-Ig plasmid is used per transfection. Each 5-mL DNA:PEI complex mixture is added to a 40-mL culture prepared previously and returned to the humidified incubator set at 130 rpm, 37°C and 5% CO_2. After 20-28 hours, 5 mL of Tryptone Feed Medium is added to each transfection and the cultures are continued for six days.

The expression profile for the DVD-Igs is shown in Table 11.

Table 11: Transient HEK293 Expression Yields of EGFR (seq. 2) Containing Antibodies and DVD-Igs

DVD ID	Sequence ID	Position	HC Linker	LC Linker	Other DVD Domain	Expression Yield (mg/L)
AB064	EGFR (seq. 2)			-	-	47.2
AB002	CD3			-	-	67.2
AB015	DLL-4			-	-	57.6
AB033	EGFR (seq. 1)			-	-	44.4
AB116	ErbB3 (seq. 3)			-	-	61.0
AB063	ErbB3 (seq. 2)			-	-	37.8
AB062	ErbB3 (seq. 1)			-	-	24.6
AB012	HGF			-	-	22.8
AB011	IGF1R			-	-	57.0
AB119	MTX (4-351-178)			-	-	1.6
AB121	NKG2D (KYK-2.0)			-	-	32.8
AB047	PlGF (ThromboGenix)			-	-	23.6
AB005	RON			-	-	67.4
AB014	VEGF (seq. 1)			-	-	52.4
AB117	VEGF (seq. 3)			-	-	70.8
AB070	VEGF (seq. 2)			-	-	1.2
DVD774	EGFR (seq. 2)	C-term	Long	Long	CD3	3.8
DVD773	EGFR (seq. 2)	N-term	Long	Long	CD3	0.6
DVD804	EGFR (seq. 2)	C-term	Long	Short	CD3	2.6
DVD803	EGFR (seq. 2)	N-term	Long	Short	CD3	40.6
DVD332	EGFR (seq. 2)	C-term	Short	Short	CD3	1.0
DVD331	EGFR (seq. 2)	N-term	Short	Short	CD3	31.0
DVD834	EGFR (seq. 2)	C-term	Short	Long	CD3	3.0
DVD833	EGFR (seq. 2)	N-term	Short	Long	CD3	1.7
DVD782	EGFR (seq. 2)	C-term	Long	Long	DLL-4	16.6
DVD781	EGFR (seq. 2)	N-term	Long	Long	DLL-4	55.0
DVD812	EGFR (seq. 2)	C-term	Long	Short	DLL-4	44.4
DVD811	EGFR (seq. 2)	N-term	Long	Short	DLL-4	43.8
DVD340	EGFR (seq. 2)	C-term	Short	Short	DLL-4	48.4
DVD339	EGFR (seq. 2)	N-term	Short	Short	DLL-4	23.6
DVD842	EGFR (seq. 2)	C-term	Short	Long	DLL-4	28.6
DVD841	EGFR (seq. 2)	N-term	Short	Long	DLL-4	48.2
DVD766	EGFR (seq. 2)	C-term	Long	Long	EGFR (seq. 1)	7.8
DVD765	EGFR (seq. 2)	N-term	Long	Long	EGFR (seq. 1)	7.0
DVD796	EGFR (seq. 2)	C-term	Long	Short	EGFR (seq. 1)	15.6
DVD795	EGFR (seq. 2)	N-term	Long	Short	EGFR (seq. 1)	26.6
DVD322	EGFR (seq. 2)	C-term	Short	Short	EGFR (seq. 1)	12.4
DVD321	EGFR (seq. 2)	N-term	Short	Short	EGFR (seq. 1)	15.6
DVD826	EGFR (seq. 2)	C-term	Short	Long	EGFR (seq. 1)	13.6
DVD825	EGFR (seq. 2)	N-term	Short	Long	EGFR (seq. 1)	12.4
DVD788	EGFR (seq. 2)	C-term	Long	Long	ErbB3 (seq. 3)	42.0
DVD787	EGFR (seq. 2)	N-term	Long	Long	ErbB3 (seq. 3)	32.8
DVD818	EGFR (seq. 2)	C-term	Long	Short	ErbB3 (seq. 3)	45.0
DVD817	EGFR (seq. 2)	N-term	Long	Short	ErbB3 (seq. 3)	29.8
DVD756	EGFR (seq. 2)	C-term	Short	Short	ErbB3 (seq. 3)	41.0
DVD755	EGFR (seq. 2)	N-term	Short	Short	ErbB3 (seq. 3)	22.4

DVD ID	Sequence ID	Position	HC Linker	LC Linker	Other DVD Domain	Expression Yield (mg/L)
DVD848	EGFR (seq. 2)	C-term	Short	Long	ErbB3 (seq. 3)	37.6
DVD847	EGFR (seq. 2)	N-term	Short	Long	ErbB3 (seq. 3)	34.0
DVD772	EGFR (seq. 2)	C-term	Long	Long	ErbB3 (seq. 2)	0.9
DVD771	EGFR (seq. 2)	N-term	Long	Long	ErbB3 (seq. 2)	15.2
DVD802	EGFR (seq. 2)	C-term	Long	Short	ErbB3 (seq. 2)	0.9
DVD801	EGFR (seq. 2)	N-term	Long	Short	ErbB3 (seq. 2)	22.4
DVD330	EGFR (seq. 2)	C-term	Short	Short	ErbB3 (seq. 2)	1.2
DVD329	EGFR (seq. 2)	N-term	Short	Short	ErbB3 (seq. 2)	16.6
DVD832	EGFR (seq. 2)	C-term	Short	Long	ErbB3 (seq. 2)	0.5
DVD831	EGFR (seq. 2)	N-term	Short	Long	ErbB3 (seq. 2)	15.0
DVD770	EGFR (seq. 2)	C-term	Long	Long	ErbB3 (seq. 1)	17.4
DVD769	EGFR (seq. 2)	N-term	Long	Long	ErbB3 (seq. 1)	16.2
DVD800	EGFR (seq. 2)	C-term	Long	Short	ErbB3 (seq. 1)	15.6
DVD799	EGFR (seq. 2)	N-term	Long	Short	ErbB3 (seq. 1)	26.4
DVD328	EGFR (seq. 2)	C-term	Short	Short	ErbB3 (seq. 1)	7.8
DVD327	EGFR (seq. 2)	N-term	Short	Short	ErbB3 (seq. 1)	10.2
DVD830	EGFR (seq. 2)	C-term	Short	Long	ErbB3 (seq. 1)	17.2
DVD829	EGFR (seq. 2)	N-term	Short	Long	ErbB3 (seq. 1)	17.2
DVD778	EGFR (seq. 2)	C-term	Long	Long	HGF	2.0
DVD777	EGFR (seq. 2)	N-term	Long	Long	HGF	24.8
DVD808	EGFR (seq. 2)	C-term	Long	Short	HGF	9.8
DVD807	EGFR (seq. 2)	N-term	Long	Short	HGF	33.6
DVD336	EGFR (seq. 2)	C-term	Short	Short	HGF	7.6
DVD335	EGFR (seq. 2)	N-term	Short	Short	HGF	23.8
DVD838	EGFR (seq. 2)	C-term	Short	Long	HGF	5.8
DVD837	EGFR (seq. 2)	N-term	Short	Long	HGF	23.4
DVD776	EGFR (seq. 2)	C-term	Long	Long	IGF1R	0.3
DVD775	EGFR (seq. 2)	N-term	Long	Long	IGF1R	23.4
DVD806	EGFR (seq. 2)	C-term	Long	Short	IGF1R	4.4
DVD805	EGFR (seq. 2)	N-term	Long	Short	IGF1R	44.8
DVD334	EGFR (seq. 2)	C-term	Short	Short	IGF1R	18.6
DVD333	EGFR (seq. 2)	N-term	Short	Short	IGF1R	48.0
DVD836	EGFR (seq. 2)	C-term	Short	Long	IGF1R	0.4
DVD835	EGFR (seq. 2)	N-term	Short	Long	IGF1R	27.8
DVD1210	EGFR (seq. 2)	C-term	Short	Short	MTX (4-351-178)	0.0
DVD1211	EGFR (seq. 2)	N-term	Short	Short	MTX (4-351-178)	1.9
DVD1214	EGFR (seq. 2)	C-term	Long	Long	NKG2D (KYK-2.0)	38.4
DVD1215	EGFR (seq. 2)	N-term	Long	Long	NKG2D (KYK-2.0)	19.6
DVD784	EGFR (seq. 2)	C-term	Long	Long	PlGF (ThromboGenix)	28.8
DVD783	EGFR (seq. 2)	N-term	Long	Long	PlGF (ThromboGenix)	23.2
DVD814	EGFR (seq. 2)	C-term	Long	Short	PlGF (ThromboGenix)	21.8
DVD813	EGFR (seq. 2)	N-term	Long	Short	PlGF (ThromboGenix)	28.2
DVD342	EGFR (seq. 2)	C-term	Short	Short	PlGF (ThromboGenix)	38.2

DVD ID	Sequence ID	Position	HC Linker	LC Linker	Other DVD Domain	Expression Yield (mg/L)
DVD341	EGFR (seq. 2)	N-term	Short	Short	PlGF (ThromboGenix)	32.2
DVD844	EGFR (seq. 2)	C-term	Short	Long	PlGF (ThromboGenix)	22.0
DVD843	EGFR (seq. 2)	N-term	Short	Long	PlGF (ThromboGenix)	24.2
DVD768	EGFR (seq. 2)	C-term	Long	Long	RON	35.6
DVD767	EGFR (seq. 2)	N-term	Long	Long	RON	44.8
DVD798	EGFR (seq. 2)	C-term	Long	Short	RON	49.0
DVD797	EGFR (seq. 2)	N-term	Long	Short	RON	68.4
DVD326	EGFR (seq. 2)	C-term	Short	Short	RON	55.6
DVD325	EGFR (seq. 2)	N-term	Short	Short	RON	32.4
DVD828	EGFR (seq. 2)	C-term	Short	Long	RON	46.6
DVD827	EGFR (seq. 2)	N-term	Short	Long	RON	52.6
DVD780	EGFR (seq. 2)	C-term	Long	Long	VEGF (seq. 1)	0.1
DVD779	EGFR (seq. 2)	N-term	Long	Long	VEGF (seq. 1)	13.2
DVD810	EGFR (seq. 2)	C-term	Long	Short	VEGF (seq. 1)	1.4
DVD809	EGFR (seq. 2)	N-term	Long	Short	VEGF (seq. 1)	17.8
DVD338	EGFR (seq. 2)	C-term	Short	Short	VEGF (seq. 1)	2.0
DVD337	EGFR (seq. 2)	N-term	Short	Short	VEGF (seq. 1)	22.0
DVD840	EGFR (seq. 2)	C-term	Short	Long	VEGF (seq. 1)	1.6
DVD839	EGFR (seq. 2)	N-term	Short	Long	VEGF (seq. 1)	17.2
DVD792	EGFR (seq. 2)	C-term	Long	Long	VEGF (seq. 3)	56.4
DVD791	EGFR (seq. 2)	N-term	Long	Long	VEGF (seq. 3)	2.4
DVD822	EGFR (seq. 2)	C-term	Long	Short	VEGF (seq. 3)	83.0
DVD821	EGFR (seq. 2)	N-term	Long	Short	VEGF (seq. 3)	34.4
DVD760	EGFR (seq. 2)	C-term	Short	Short	VEGF (seq. 3)	74.4
DVD759	EGFR (seq. 2)	N-term	Short	Short	VEGF (seq. 3)	50.4
DVD852	EGFR (seq. 2)	C-term	Short	Long	VEGF (seq. 3)	71.6
DVD851	EGFR (seq. 2)	N-term	Short	Long	VEGF (seq. 3)	50.2
DVD790	EGFR (seq. 2)	C-term	Long	Long	VEGF (seq. 2)	1.3
DVD789	EGFR (seq. 2)	N-term	Long	Long	VEGF (seq. 2)	46.0
DVD820	EGFR (seq. 2)	C-term	Long	Short	VEGF (seq. 2)	1.6
DVD819	EGFR (seq. 2)	N-term	Long	Short	VEGF (seq. 2)	57.0
DVD758	EGFR (seq. 2)	C-term	Short	Short	VEGF (seq. 2)	1.1
DVD757	EGFR (seq. 2)	N-term	Short	Short	VEGF (seq. 2)	52.4
DVD850	EGFR (seq. 2)	C-term	Short	Long	VEGF (seq. 2)	2.4
DVD849	EGFR (seq. 2)	N-term	Short	Long	VEGF (seq. 2)	49.8

All DVD-Igs expressed well in 293 cells. DVD-Igs could be easily purified over a protein A column. In most cases >5 mg/L purified DVD-Ig could be obtained easily from supernatants of 293 cells.

Example 1.4.5: Characterization and lead selection of A/B DVD-Igs

The binding affinities of anti-A/B DVD-Igs are analyzed on Biacore against both protein A and protein B. The tetravalent property of the DVD-Ig is examined by multiple binding studies on Biacore. Meanwhile, the neutralization potency of the DVD-Igs for protein A and protein B are assessed by bioassays, respectively, as described herein. The DVD-Ig molecules that best retain the affinity and potency of the original parental mAbs are selected for in-depth physicochemical and bio-analytical (rat PK) characterizations as described herein for each mAb. Based on the collection of analyses, the final lead DVD-Ig is advanced into CHO stable cell line development, and the CHO-derived material is employed in stability, pharmacokinetic and efficacy studies in cynomolgus monkey, and preformulation activities.

Example 2: Generation and Characterization of Dual Variable Domain Immunoglobulins (DVD-Ig)

Dual variable domain immunoglobulins (DVD-Ig) using parent antibodies with known amino acid sequences were generated by synthesizing polynucleotide fragments encoding DVD-Ig variable heavy and DVD-Ig variable light chain sequences and cloning the fragments into a pHybC-D2 vector according to Example 1.4.4.1. The DVD-Ig contructs were cloned into and expressed in 293 cells as described in Example 1,4.4.2. The DVD-Ig protein was purified according to standard methods. Functional characteristics were determined according to the methods described in Example 1.1.1 and 1.1.2 as indicated.

The following examples each comprise a table that contains the sequences of the DVD-Ig VH and VL chains.

Example 2.1: Generation of EGFR (seq. 2) and EGFR (seq. 1) DVD-Igs with Linker Set 1

Table 12

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
59	DVD321H	AB064VH	AB033VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**QVQLKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWS GGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSN DTAIYYCARALTYYDYEFAYWGQGTLVTVSA
60	DVD321L	AB064VL	AB033VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DILLTQSPVILSVSPGERVSFSCRASQ SIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTT FGAGTKLELKR
61	DVD322H	AB033VH	AB064VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE FAYWGQGTLVTVSA**ASTKGP**QVQLQESGPGLVKPS QTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMG YISYSGNTRYQPSLKSRITISRDTSKNQFFLKLNS VTAADTATYYCVTAGRGFPYWGQGTLVTVSS
62	DVD322L	AB033VL	AB064VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

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Example 2.2: Generation of EGFR (seq. 2) and EGFR (seq. 1) DVD-Igs with Linker Set 2

Table 13

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
63	DVD765H	AB064VH	AB033VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**QVQLKQSGPGL VQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLE WLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFK MNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVT VSA
64	DVD765L	AB064VL	AB033VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DILLTQSPVILSVSPGERVS FSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESI SGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQ NNNWPTTFGAGTKLELKR
65	DVD766H	AB033VH	AB064VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE FAYWGQGTLVTVSA**ASTKGPSVFPLAP**QVQLQESG PGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPG KGLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQ FFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVT VSS
66	DVD766L	AB033VL	AB064VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

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Example 2.3: Generation of EGFR (seq. 2) and EGFR (seq. 1) DVD-Igs with Linker Set 3

Table 14

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
67	DVD795H	AB064VH	AB033VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**QVQLKQSGPGL VQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLE WLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFK MNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVT VSA
68	DVD795L	AB064VL	AB033VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DILLTQSPVILSVSPGERVSFSCRASQ SIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTT FGAGTKLELKR
69	DVD796H	AB033VH	AB064VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE FAYWGQGTLVTVSA**ASTKGPSVFPLAP**QVQLQESG PGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPG KGLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQ FFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVT VSS
70	DVD796H	AB033VL	AB064VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

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Example 2.4: Generation of EGFR (seq. 2) and EGFR (seq. 1) DVD-Igs with Linker Set 4

Table 15

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
71	DVD825H	AB064VH	AB033VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**QVQLKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWS GGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSN DTAIYYCARALTYYDYEFAYWGQGTLVTVSA
72	DVD825L	AB064VL	AB033VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DILLTQSPVILSVSPGERVS FSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESI SGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQ NNNWPTTFGAGTKLELKR
73	DVD826H	AB033VH	AB064VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE FAYWGQGTLVTVSA**ASTKGP**QVQLQESGPGLVKPS QTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMG YISYSGNTRYQPSLKSRITISRDTSKNQFFLKLNS VTAADTATYYCVTAGRGFPYWGQGTLVTVSS
74	DVD826L	AB033VL	AB064VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

Example 2.5: Generation of EGFR (seq. 2) and RON DVD-Igs with Linker Set 1

Table 16

SEQ ID No.	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
75	DVD325H	AB064VH	AB005VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVQSGGGLVKPGGSL RLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISY DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYCARFSGWPNNYYYYGMDVWGQGTTVTVS S
76	DVD325L	AB064VL	AB005VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DVVMTQSPLSLPVTPGEPASISCRSSQ SLLHSNGFNYVDWYLQKPGQSPHLLIYFGSYRASG VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQAL QTPPWTFGQGTKVEIRR
77	DVD326H	AB005VH	AB064VH	EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYAMH WVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARFSGWPNN YYYYGMDVWGQGTTVTVSS**ASTKGP**QVQLQESGPG LVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKG LEWMGYISYSGNTRYQPSLKSRITISRDTSKNQFF LKLNSVTAADTATYYCVTAGRGFPYWGQGTLVTVS S
78	DVD326L	AB005VL	AB064VL	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGF NYVDWYLQKPGQSPHLLIYFGSYRASGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCMQALQTPPWTFG QGTKVEIRR**TVAAP**DIQMTQSPSSMSVSVGDRVTI TCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDD GVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQY AQFPWTFGGGTKLEIKR

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Example 2.6: Generation of EGFR (seq. 2) and RON DVD-Igs with Linker Set 2

Table 17

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
79	DVD767H	AB064VH	AB005VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVQSGGGL VKPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLE WVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCARFSGWPNNYYYYGMDVWGQ GTTVTVSS
80	DVD767L	AB064VL	AB005VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DVVMTQSPLSLPVTPGEPAS ISCRSSQSLLHSNGFNYVDWYLQKPGQSPHLLIYF GSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCMQALQTPPWTFGQGTKVEIRR
81	DVD768H	AB005VH	AB064VH	EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYAMH WVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARFSGWPNN YYYYGMDVWGQGTTVTVSS**ASTKGPSVFPLAP**QVQ LQESGPGLVKPSQTLSLTCTVSGYSISSDFAWNWI RQPPGKGLEWMGYISYSGNTRYQPSLKSRITISRD TSKNQFFLKLNSVTAADTATYYCVTAGRGFPYWGQ GTLVTVSS
82	DVD768L	AB005VL	AB064VL	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGF NYVDWYLQKPGQSPHLLIYFGSYRASGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCMQALQTPPWTFG QGTKVEIRR**TVAAPSVFIFPP**DIQMTQSPSSMSVS VGDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIY HGTNLDDGVPSRFSGSGSGTDYTLTISSLQPEDFA TYYCVQYAQFPWTFGGGTKLEIKR

Example 2.7: Generation of EGFR (seq. 2) and RON DVD-Igs with Linker Set 3

Table 18

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
83	DVD797H	AB064VH	AB005VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVQSGGGL VKPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLE WVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCARFSGWPNNYYYYGMDVWGQ GTTVTVSS
84	DVD797L	AB064VL	AB005VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DVVMTQSPLSLPVTPGEPASISCRSSQ SLLHSNGFNYVDWYLQKPGQSPHLLIYFGSYRASG VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQAL QTPPWTFGQGTKVEIRR
85	DVD798H	AB005VH	AB064VH	EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYAMH WVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARFSGWPNN YYYYGMDVWGQGTTVTVSSA**STKGPSVFPLAP**QVQ LQESGPGLVKPSQTLSLTCTVSGYSISSDFAWNWI RQPPGKGLEWMGYISYSGNTRYQPSLKSRITISRD TSKNQFFLKLNSVTAADTATYYCVTAGRGFPYWGQ GTLVTVSS
86	DVD798L	AB005VL	AB064VL	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGF NYVDWYLQKPGQSPHLLIYFGSYRASGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCMQALQTPPWTFG QGTKVEIRR**TVAAP**DIQMTQSPSSMSVSVGDRVTI TCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDD GVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQY AQFPWTFGGGTKLEIKR

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Example 2.8: Generation of EGFR (seq. 2) and RON DVD-Igs with Linker Set 4

Table 19

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
87	DVD827H	AB064VH	AB005VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVQSGGGLVKPGGSL RLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISY DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYCARFSGWPNNYYYYGMDVWGQGTTVTVS S
88	DVD827L	AB064VL	AB005VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DVVMTQSPLSLPVTPGEPAS ISCRSSQSLLHSNGFNYVDWYLQKPGQSPHLLIYF GSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCMQALQTPPWTFGQGTKVEIRR
89	DVD828H	AB005VH	AB064VH	EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYAME WVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARFSGWPNN YYYYGMDVWGQGTTVTVSS**ASTKGP**QVQLQESGPG LVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKG LEWMGYISYSGNTRYQPSLKSRITISRDTSKNQFF LKLNSVTAADTATYYCVTAGRGFPYWGQGTLVTVS S
90	DVD828L	AB005VL	AB064VL	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGF NYVDWYLQKPGQSPHLLIYFGSYRASGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCMQALQTPPWTFG QGTKVEIRR**TVAAPSVFIFPP**DIQMTQSPSSMSVS VGDRVTITCHSSQDINSNIGWLQQKPGKSPKGLIY HGTNLDDGVPSRFSGSGSGTDYTLTISSLQPEDFA TYYCVQYAQFPWTFGGGTKLEIKR

Example 2.9: Generation of EGFR (seq. 2) and ErbB3 (seq. 1) DVD-Igs with Linker Set 1

Table 20

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
91	DVD327H	AB064VH	AB062VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**QVQLQQWGAGLLKPSETL SLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINH SGSTNYNPSLKSRVTISVETSKNQFSLKLSSVTAA DTAVYYCARDKWTWYFDLWGRGTLVTVSS
92	DVD327L	AB064VL	AB062VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIEMTQSPDSLAVSLGERATINCRSSQ SVLYSSSNRNYLAWYQQNPGQPPKLLIYWASTRES GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQY YSTPRTFGQGTKVEIKR
93	DVD328H	AB062VH	AB064VH	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWS WIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTIS VETSKNQFSLKLSSVTAADTAVYYCARDKWTWYFD LWGRGTLVTVSS**ASTKGP**QVQLQESGPGLVKPSQT LSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMGYI SYSGNTRYQPSLKSRITISRDTSKNQFFLKLNSVT AADTATYYCVIAGRGFPYWGQGTLVTVSS
94	DVD328L	AB062VL	AB064VL	DIEMTQSPDSLAVSLGERATINCRSSQSVLYSSSN RNYLAWYQQNPGQPPKLLIYWASTRESGVPDRFSG SGSGTDFTLTISSLQAEDVAVYYCQQYYSTPRTFG QGTKVEIKR**TVAAP**DIQMTQSPSSMSVSVGDRVTI TCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDD GVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQY AQFPWTFGGGTKLEIKR

Example 2.10: Generation of EGFR (seq. 2) and ErbB3 (seq. 1) DVD-Igs with Linker Set 2

Table 21

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
95	DVD769H	AB064VH	AB062VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQQWGAGL LKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLE WIGEINHSGSTNYNPSLKSRVTISVETSKNQFSLK LSSVTAADTAVYYCARDKWTWYFDLWGRGTLVTVS S
96	DVD768L	AB064VL	AB062VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIEMTQSPDSLAVSLGERAT INCRSSQSVLYSSSNRNYLAWYQQNPGQPPKLLIY WASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVA VYYCQQYYSTPRTFGQGTKVEIKR
97	DVD770H	AB062VH	AB064VH	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWS WIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTIS VETSKNQFSLKLSSVTAADTAVYYCARDKWTWYFD LWGRGTLVTVSS**ASTKGPSVFPLAP**QVQLQESGPG LVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKG LEWMGYISYSGNTRYQPSLKSRITISRDTSKNQFF LKLNSVTAADTATYYCVTAGRGFPYWGQGTLVTVS S
98	DVD770L	AB062VL	AB064VL	DIEMTQSPDSLAVSLGERATINCRSSQSVLYSSSN RNYLAWYQQNPGQPPKLLIYWASTRESGVPDRFSG SGSGTDFTLTISSLQAEDVAVYYCQQYYSTPRTFG QGTKVEIKR**TVAAPSVFIFPP**DIQMTQSPSSMSVS VGDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIY HGTNLDDGVPSRFSGSGSGTDYTLTISSLQPEDFA TYYCVQYAQFPWTFGGGTKLEIKR

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Example 2.11: *Generation of EGFR (seq. 2) and ErbB3 (seq. 1) DVD-Igs with Linker Set 3*

Table 22

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
99	DVD799H	AB064VH	AB062VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQQWGAGL LKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLE WIGEINHSGSTNYNPSLKSRVTISVETSKNQFSLK LSSVTAADTAVYYCARDKWTWYFDLWGRGTLVTVS S
100	DVD799L	AB064VL	AB062VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIEMTQSPDSLAVSLGERATINCRSSQ SVLYSSSNRNYLAWYQQNPGQPPKLLIYWASTRES GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQY *YSTPRTFGQGTKVEIKR*
101	DVD800H	AB062VH	AB064VH	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWS WIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTIS VETSKNQFSLKLSSVTAADTAVYYCARDKWTWYFD LWGRGTLVTVSS**ASTKGPSVFPLAP**QVQLQESGPG LVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKG LEWMGYISYSGNTRYQPSLKSRITISRDTSKNQFF LKLNSVTAADTATYYCVTAGRGFPYWGQGTLVTVS S
102	DVD800L	AB062VL	AB064VL	DIEMTQSPDSLAVSLGERATINCRSSQSVLYSSSN RNYLAWYQQNPGQPPKLLIYWASTRESGVPDRFSG SGSGTDFTLTISSLQAEDVAVYYCQQYYSTPRTFG QGTKVEIKR**TVAAP**DIQMTQSPSSMSVSVGDRVTI TCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDD GVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQY AQFPWTFGGGTKLSIKR

Example 2.12: Generation of EGFR (seq. 2) and ErbB3 (seq. 1) DVD-Igs with Linker Set 4

Table 23

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
103	DVD829H	AB064VH	AB062VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**QVQLQQWGAGLLKPSETL SLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINH SGSTNYNPSLKSRVTISVETSKNQFSLKLSSVTAA DTAVYYCARDKWTWYFDLWGRGTLVTVSS
104	DVD829L	AB064VL	AB062VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIEMTQSPDSLAVSLGERAT INCRSSQSVLYSSSNRNYLAWYQQNPGQPPKLLIY WASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVA VYYCQQYYSTPRTFGQGTKVEIKR
105	DVD830H	AB062VH	AB064VH	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWS WIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTIS VETSKNQFSLKLSSVTAADTAVYYCARDKWTWYFD LWGRGTLVTVSS**ASTKGP**QVQLQESGPGLVKPSQT LSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMGYI SYSGNTRYQPSLKSRITISRDTSKNQFFLKLNSVT AADTATYYCVTAGRGFPYWGQGTLVTVSS
106	DVD830L	AB062VL	AB064VL	DIEMTQSPDSLAVSLGERATINCRSSQSVLYSSSN RNYLAWYQQNPGQPPKLLIYWASTRESGVPDRFSG SGSGTDFTLTISSLQAEDVAVYYCQQYYSTPRTFG QGTKVEIKR**TVAAPSVFIFPP**DIQMTQSPSSMSVS VGDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIY HGTNLDDGVPSRFSGSGSGTDYTLTISSLQPEDFA TYYCVQYAQFPWTFGGGTKLEIKR

Example 2.13: Generation of EGFR (seq. 2) and ErbB3 (seq. 2) DVD-Igs with Linker Set 1

Table 24

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
107	DVD329H	AB064VH	AB063VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGFTFSIYSMNWVRQAPGKGLEWVSYISS SSSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRD EDTAVYYCARDRGDFDAFDIWGQGTMVTVSS
108	DVD329L	AB064VL	AB063VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCQASQ DITNYLNWYQQKPGKAPKLLIYDASNLETGVPSRF SGSGSGTDFTFTISSLQPEDIATYNCQQCENFPIT PGQGTRLEIKR
109	DVD330H	AB063VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSIYSMN WVRQAPGKGLEWVSYISSSSSTIYYADSVKGRFTI SRDNAKNSLYLQMNSLRDEDTAVYYCARDRGDFDA FDIWGQGTMVTVSS**ASTKGP**QVQLQESGPGLVKPS QTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMG YISYSGNTRYQPSLKSRITISRDTSKNQFFLKLNS VTAADTATYYCVTAGRGFPYWGQGTLVTVSS
110	DVD330L	AB063VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNW YQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTD FTFTISSLQPEDIATYNCQQCENFPITFGQGTRLE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

Example 2.14: Generation of EGFR (seq. 2) and ErbB3 (seq. 2) DVD-Igs with Linker Set 2

Table 25

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
111	DVD771H	AB064VH	AB063VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVESGGGL VQPGGSLRLSCAASGFTFSIYSMNWVRQAPGKGLE WVSYISSSSSTIYYADSVKGRFTISRDNAKNSLYL QMNSLRDEDTAVYYCARDRGDFDAFDIWGQGTMVT VSS
112	DVD771L	AB064VL	AB063VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCQASQDITNYLNWYQQKPGKAPKLLIYDASNLE TGVPSRFSGSGSGTDFTFTISSLQPEDIATYNCQQ CENFPITFGQGTRLEIKR
113	DVD772H	AB063VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSIYSMN WVRQAPGKGLEWVSYISSSSSTIYYADSVKGRFTI SRDNAKNSLYLQMNSLRDEDTAVYYCARDRGDFDA FDIWGQGTMVTVSS**ASTKGPSVFPLAP**QVQLQESG PGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPG KGLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQ FFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVT VSS
114	DVD772L	AB063VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNW YQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTD FTFTISSLQPEDIATYNCQQCENFPITFGQGTRLE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

Example 2.15: Generation of EGFR (seq. 2) and ErbB3 (seq. 2) DVD-Igs with Linker Set 3

Table 26

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 123456789012345678901234567890 12345
115	DVD801H	AB064VH	AB063VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVESGGGL VQPGGSLRLSCAASGFTFSIYSMNWVRQAPGKGLE WVSYISSSSSTIYYADSVKGRFTISRDNAKNSLYL QMNSLRDEDTAVYYCARDRGDFDAFDIWGQGTMVT VSS
116	DVD801L	AB064VL	AB063VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCQASQ DITNYLNWYQQKPGKAPKLLIYDASNLETGVPSRF SGSGSGTDFTFTISSLQPEDIATYNCQQCENFPIT FGQGTRLEIKR
117	DVD802H	AB063VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSIYSMN WVRQAPGKGLEWVSYISSSSSTIYYADSVKGRFTI SRDNAKNSLYLQMNSLRDEDTAVYYCARDRGDFDA FDIWGQGTMVTVSS**ASTKGPSVFPLAP**QVQLQESG PGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPG KGLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQ FFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVT VSS
118	DVD802L	AB063VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNW YQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTD FTFTISSLQPEDIATYNCQQCENFPITFGQGTRLE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

Example 2.16: Generation of EGFR (seq. 2) and ErbB3 (seq. 2) DVD-Igs with Linker Set 4

Table 27

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
119	DVD831H	AB064VH	AB063VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGFTFSIYSMNWVRQAPGKGLEWVSYISS SSSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRD EDTAVYYCARDRGDFDAFDIWGQGTMVTVSS
120	DVD831L	AB064VL	AB063VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCQASQDITNYLNWYQQKPGKAPKLLIYDASNLE TGVPSRFSGSGSGTDFTFTISSLQPEDIATYNCQQ CENFPITFGQGTRLEIKR
121	DVD832H	AB063VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSIYSMN WVRQAPGKGLEWVSYISSSSSTIYYADSVKGRFTI SRDNAKNSLYLQMNSLRDEDTAVYYCARDRGDFDA FDIWGQGTMVTVSS**ASTKGP**QVQLQESGPGLVKPS QTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMG YISYSGNTRYQPSLKSRITISRDTSKNQFFLKLNS VTAADTATYYCVTAGRGFPYWGQGTLVTVSS
122	DVD832L	AB063VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNW YQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTD FTFTISSLQPEDIATYNCQQCENFPITFGQGTRLE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

Example 2.17: Generation of EGFR (seq. 2) and CD3 DVD-Igs with Linker Set 1

Table 28

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 123456789012345678901234567890123 45
123	DVD331H	AB064VH	AB002VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**QVQLQQSGAELARPGASV KMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINP SRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTS EDSAVYYCARYYDDHYCLDYWGQGTTLTVSS
124	DVD331L	AB064VL	AB002VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**QIVLTQSPAIMSASPGEKVTMTCRASS SVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFS GSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTF GSGTKLEINR
125	DVD332H	AB002VH	AB064VH	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMH WVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATL TTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYC LDYWGQGTTLTVSS**ASTKGP**QVQLQESGPGLVKPS QTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMG YISYSGNTRYQPSLKSRITISRDTSKNQFFLKLNS VTAADTATYYCVTAGRGFPYWGQGTLVTVSS
126	DVD332L	AB002VL	AB064VL	QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWY QQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSY SLTISSMEAEDAATYYCQQWSSNPLTFGSGTKLEI NR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQD INSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRFS GSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWTF GGGTKLEIKR

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Example 2.18: Generation of EGFR (seq. 2) and CD3 DVD-Igs with Linker Set 2

Table 29

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
127	DVD773H	AB064VH	AB002VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQQSGAEL ARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLE WIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYM QLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLT VSS
128	DVD773L	AB064VL	AB002VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**QIVLTQSPAIMSASPGEKVT MTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVAS GVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQW SSNPLTFGSGTKLEINR
129	DVD774H	AB002VH	AB064VH	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMH WVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATL TTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYC LDYWGQGTTLTVSS**ASTKGPSVFPLAP**QVQLQESG PGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPG KGLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQ FFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVT VSS
130	DVD774L	AB002VL	AB064VL	QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWY QQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSY SLTISSMEAEDAATYYCQQWSSNPLTFGSGTKLEI NR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVTI TCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDD GVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQY AQFPWTFGGGTKLEIKR

Example 2.19: Generation of EGFR (seq. 2) and CD3 DVD-Igs with Linker Set 3

Table 30

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
131	DVD803H	AB064VH	AB002VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQQSGAEL ARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLE WIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYM QLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLT VSS
132	DVD803L	AB064VL	AB002VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**QIVLTQSPAIMSASPGEKVTMTCRASS SVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFS GSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTF GSGTKLEINR
133	DVD804H	AB002VH	AB064VH	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMH WVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATL TTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYC LDYWGQGTTLTVSS**ASTKGPSVFPLAP**QVQLQESG PGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPG KGLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQ FFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVT VSS
134	DVD804L	AB002VL	AB064VL	QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWY QQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSY SLTISSMEAEDAATYYCQQWSSNPLTFGSGTKLEI NR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQD INSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRFS GSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWTF GGGTKLEIKR

Example 2.20: Generation of EGFR (seq. 2) and CD3 DVD-Igs with Linker Set 4

Table 31

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
135	DVD833H	AB064VH	AB002VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**QVQLQQSGAELARPGASV KMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINP SRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTS EDSAVYYCARYYDDHYCLDYWGQGTTLTVSS
136	DVD833L	AB064VL	AB002VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**QIVLTQSPAIMSASPGEKVT MTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVAS GVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQW SSNPLTFGSGTKLEINR
137	DVD834H	AB002VH	AB064VH	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMH WVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATL TTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYC LDYWGQGTTLTVSS**ASTKGP**QVQLQESGPGLVKPS QTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMG YISYSGNTRYQPSLKSRITISRDTSKNQFFLKLNS VTAADTATYYCVTAGRGFPYWGQGTLVTVSS
138	DVD834L	AB002VL	AB064VL	QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWY QQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSY SLTISSMEAEDAATYYCQQWSSNPLTFGSGTKLEI NR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVTI TCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDD GVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQY AQFPWTFGGGTKLEIKR

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Example 2.21: Generation of EGFR (seq. 2) and IGF1R DVD-Igs with Linker Set 1

Table 32

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
139	DVD333H	AB064VH	AB011VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLLESGGGLVQPGGSL RLSCTASGFTFSSYAMNWVRQAPGKGLEWVSAISG SGGTTFYADSVKGRFTISRDNSRTTLYLQMNSLRA EDTAVYYCAKDLGWSDSYYYYYGMDVWGQGTTVTV SS
140	DVD333L	AB064VL	AB011VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQFPSSLSASVGDRVTITCRASQ GIRNDLGWYQQKPGKAPKRLIYAASRLHRGVPSRF SGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPCS FGQGTKLEIKR
141	DVD334H	AB011VH	AB064VH	EVQLLESGGGLVQPGGSLRLSCTASGFTFSSYAMN WVRQAPGKGLEWVSAISGSGGTTFYADSVKGRFTI SRDNSRTTLYLQMNSLRAEDTAVYYCAKDLGWSDS YYYYYGMDVWGQGTTVTVSS**ASTKGP**QVQLQESGP GLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGK GLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQF FLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVTV SS
142	DVD334L	AB011VL	AB064VL	DIQMTQFPSSLSASVGDRVTITCRASQGIRNDLGW YQQKPGKAPKRLIYAASRLHRGVPSRFSGSGSGTE FTLTISSLQPEDFATYYCLQHNSYPCSFGQGTKLE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

Example 2.22: Generation of EGFR (seq. 2) and IGF1R DVD-Igs with Linker Set 2

Table 33

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
143	DVD775H	AB064VH	AB011VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLLESGGGL VQPGGSLRLSCTASGFTFSSYAMNWVRQAPGKGLE WVSAISGSGGTTFYADSVKGRFTISRDNSRTTLYL QMNSLRAEDTAVYYCAKDLGWSDSYYYYGMDVWG QGTTVTVSS
144	DVD775L	AB064VL	AB011VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQFPSSLSASVGDRVT ITCRASQGIRNDLGWYQQKPGKAPKRLIYAASRLH RGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQ HNSYPCSFGQGTKLEIKR
145	DVD776H	AB011VH	AB064VH	EVQLLESGGGLVQPGGSLRLSCTASGFTFSSYAMN WVRQAPGKGLEWVSAISGSGGTTFYADSVKGRFTI SRDNSRTTLYLQMNSLRAEDTAVYYCAKDLGWSDS YYYYYGMDVWGQGTTVTVSS**ASTKGPSVFPLAP**QV QLQESGPGLVKPSQTLSLTCTVSGYSISSDFAWNW IRQPPGKGLEWMGYISYSGNTRYQPSLKSRITISR DTSKNQFFLKLNSVTAADTATYYCVTAGRGFPYWG QGTLVTVSS
146	DVD776L	AB011VL	AB064VL	DIQMTQFPSSLSASVGDRVTITCRASQGIRNDLGW YQQKPGKAPKRLIYAASRLHRGVPSRFSGSGSGTE FTLTISSLQPEDFATYYCLQHNSYPCSFGQGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

Example 2.23: Generation of EGFR (seq. 2) and IGF1R DVD-Igs with Linker Set 3

Table 34

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
147	DVD805H	AB064VH	AB011VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLLESGGGL VQPGGSLRLSCTASGFTFSSYAMNWVRQAPGKGLE WVSAISGSGGTTFYADSVKGRFTISRDNSRTTLYL QMNSLRAEDTAVYYCAKDLGWSDSYYYYYGMDVWG QGTTVTVSS
148	DVD805L	AB064VL	AB011VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQFPSSLSASVGDRVTITCRASQ GIRNDLGWYQQKPGKAPKRLIYAASRLHRGVPSRF SGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPCS FGQGTKLEIKR
149	DVD806H	AB011VH	AB064VH	EVQLLESGGGLVQPGGSLRLSCTASGFTFSSYAMN WVRQAPGKGLEWVSAISGSGGTTFYADSVKGRFTI SRDNSRTTLYLQMNSLRAEDTAVYYCAKDLGWSDS YYYYYGMDVWGQGTTVTVSS**ASTKGPSVFPLAP**QV QLQESGPGLVKPSQTLSLTCTVSGYSISSDFAWNW IRQPPGKGLEWMGYISYSGNTRYQPSLKSRITISR DTSKNQFFLKLNSVTAADTATYYCVTAGRGFPYWG QGTLVTVSS
150	DVD806L	AB011VL	AB064VL	DIQMTQFPSSLSASVGDRVTITCRASQGIRNDLGW YQQKPGKAPKRLIYAASRLHRGVPSRFSGSGSGTE FTLTISSLQPEDFATYYCLQHNSYPCSFGQGTKLE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

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Example 2.24: Generation of EGFR (seq. 2) and IGF1R DVD-Igs with Linker Set 4

Table 35

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
151	DVD835H	AB064VH	AB011VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLLESGGGLVQPGGSL RLSCTASGFTFSSYAMNWVRQAPGKGLEWVSAISG SGGTTFYADSVKGRFTISRDNSRTTLYLQMNSLRA EDTAVYYCAKDLGWSDSYYYYYGMDVWGQGTTVTV SS
152	DVD835L	AB064VL	AB011VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQFPSSLSASVGDRVT ITCRASQGIRNDLGWYQQKPGKAPKRLIYAASRLH RGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQ HNSYPCSFGQGTKLEIKR
153	DVD836H	AB011VH	AB064VH	EVQLLESGGGLVQPGGSLRLSCTASGFTFSSYAMN WVRQAPGKGLEWVSAISGSGGTTFYADSVKGRFTI SRDNSRTTLYLQMNSLRAEDTAVYYCAKDLGWSDS YYYYYGMDVWGQGTTVTVSS**ASTKGP**QVQLQESGP GLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGK GLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQF FLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVTV SS
154	DVD836L	AB011VL	AB064VL	DIQMTQFPSSLSASVGDRVTITCRASQGIRNDLGW YQQKPGKAPKRLIYAASRLHRGVPSRFSGSGSGTE FTLTISSLQPEDFATYYCLQHNSYPCSFGQGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

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Example 2.25: Generation of EGFR (seq. 2) and HGF DVD-Igs with Linker Set 1

Table 36

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
155	DVD335H	AB064VH	AB012VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**QVQLVESGGGLVKPGGSL RLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISS SGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARDEYNSGWYVLFDYWGQGTLVTVSS
156	DVD335L	AB064VL	AB012VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSVSASVGDRVTITCRASQ GISSWLAWYQQKPGKAPNLLIYEASSLQSGVPSRF GGSGSGTDFTLTISSLQPEDFATYYCQQANGFPWT FGQGTKVEIKR
157	DVD336H	AB012VH	AB064VH	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCARDEYNSGW YVLFDYWGQGTLVTVSS**ASTKGP**QVQLQESGPGLV KPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKGLE WMGYISYSGNTRYQPSLKSRITISRDTSKNQFFLK LNSVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
158	DVD336L	AB012VL	AB064VL	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAW YQQKPGKAPNLLIYEASSLQSGVPSRFGGSGSGTD FTLTISSLQPEDFATYYCQQANGFPWTFGQGTKVE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

Example 2.26: Generation of EGFR (seq. 2) and HGF DVD-Igs with Linker Set 2

Table 37

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
159	DVD777H	AB064VH	AB012VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**QVQLVESGGGL VKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLE WVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYL QMNSLRAEDTAVYYCARDEYNSGWYVLFDYWGQGT LVTVSS
160	DVD777L	AB064VL	AB012VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSVSASVGDRVT ITCRASQGISSWLAWYQQKPGKAPNLLIYEASSLQ SGVPSRFGGSGSGTDFTLTISSLQPEDFATYYCQQ ANGFPWTFGQGTKVEIKR
161	DVD778H	AB012VH	AB064VH	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCARDEYNSGW YVLFDYWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQ ESGPGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQ PPGKGLEWMGYISYSGNTRYQPSLKSRITISRDTS KNQFFLKLNSVTAADTATYYCVTAGRGFPYWGQGT LVTVSS
162	DVD778L	AB012VL	AB064VL	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAW YQQKPGKAPNLLIYEASSLQSGVPSRFGGSGSGTD FTLTISSLQPEDFATYYCQQANGFPWTFGQGTKVE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

Example 2.27: Generation of EGFR (seq. 2) and HGF DVD-Igs with Linker Set 3

Table 38

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
163	DVD807H	AB064VH	AB012VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**QVQLVESGGGL VKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLE WVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYL QMNSLRAEDTAVYYCARDEYNSGWYVLFDYWGQGT LVTVSS
164	DVD807L	AB064VL	AB012VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSVSASVGDRVTITCRASQ GISSWLAWYQQKPGKAPNLLIYEASSLQSGVPSRF GGSGSGTDFTLTISSLQPEDFATYYCQQANGFPWT FGQGTKVEIKR
165	DVD808H	AB012VH	AB064VH	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCARDEYNSGW YVLFDYWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQ ESGPGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQ PPGKGLEWMGYISYSGNTRYQPSLKSRITISRDTS KNQFFLKLNSVTAADTATYYCVTAGRGFPYWGQGT LVTVSS
166	DVD808L	AB012VL	AB064VL	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAW YQQKPGKAPNLLIYEASSLQSGVPSRFGGSGSGTD FTLTISSLQPEDFATYYCQQANGFPWTFGQGTKVE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

Example 2.28: Generation of EGFR (seq. 2) and HGF DVD-Igs with Linker Set 4

Table 39

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
167	DVD837H	AB064VH	AB012VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**QVQLVESGGGLVKPGGSL RLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISS SGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARDEYNSGWYVLFDYWGQGTLVTVSS
168	DVD837L	AB064VL	AB012VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSVSASVGDRVT ITCRASQGISSWLAWYQQKPGKAPNLLIYEASSLQ SGVPSRFGGSGSGTDFTLTISSLQPEDFATYYCQQ ANGFPWTFGQGTKVEIKR
169	DVD838H	AB012VH	AB064VH	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCARDEYNSGW YVLFDYWGQGTLVTVSS**ASTKGP**QVQLQESGPGLV KPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKGLE WMGYISYSGNTRYQPSLKSRITISRDTSKNQFFLK LNSVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
170	DVD838L	AB012VL	AB064VL	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAW YQQKPGKAPNLLIYEASSLQSGVPSRFGGSGSGTD FTLTISSLQPEDFATYYCQQANGFPWTFGQGTKVE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

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Example 2.29: Generation of EGFR (seq. 2) and VEGF (seq. 1) DVD-Igs with Linker Set 1

Table 40

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
171	**DVD337H**	AB064VH	AB014VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINT YTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRA EDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSS
172	DVD337L	AB064VL	AB014VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCSASQ DISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWT FGQGTKVEIKR
173	**DVD338H**	AB014VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMN WVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGS SHWYFDVWGQGTLVTVSS**ASTKGP**QVQLQESGPGL VKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKGL EWMGYISYSGNTRYQPSLKSRITISRDTSKNQFFL KLNSVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
174	DVD338L	AB014VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNW YQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

Example 2.30: Generation of EGFR (seq. 2) and VEGF (seq. 1) DVD-Igs with Linker Set 2

Table 41

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
175	DVD779H	AB064VH	AB014VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVESGGGL VQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLE WVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYL QMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQG TLVTVSS
176	DVD779L	AB064VL	AB014VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLH SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ YSTVPWTFGQGTKVEIKR
177	*DVD780H*	*AB014VH*	*AB064VH*	*EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMN* WVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGS SHWYFDVWGQGTLVTVSS**ASTKGPSVFPLAP**QVQL QESGPGLVKPSQTLSLTCTVSGYSISSDFAWNWIR QPPGKGLEWMGYISYSGNTRYQPSLKSRITISRDT SKNQFFLKLNSVTAADTATYYCVTAGRGFPYWGQG TLVTVSS
178	DVD780L	AB014VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNW YQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

Example 2.31: Generation of EGFR (seq. 2) and VEGF (seq. 1) DVD-Igs with Linker Set 3

Table 42

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
179	DVD809H	AB064VH	AB014VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVESGGGL VQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLE WVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYL QMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQG TLVTVSS
180	DVD809L	AB064VL	AB014VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCSASQ DISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWT FGQGTKVEIKR
181	DVD810H	AB014VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMN WVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGS SHWYFDVWGQGTLVTVSS**ASTKGPSVFPLAP**QVQL QESGPGLVKPSQTLSLTCTVSGYSISSDFAWNWIR QPPGKGLEWMGYISYSGNTRYQPSLKSRITISRDT SKNQFFLKLNSVTAADTATYYCVTAGRGFPYWGQG TLVTVSS
182	DVD810L	AB014VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNW YQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

Example 2.32: Generation of EGFR (seq. 2) and VEGF (seq. 1) DVD-Igs with Linker Set 4

Table 43

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
183	DVD839H	AB064VH	AB014VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINT YTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRA EDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSS
184	DVD839L	AB064VL	AB014VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLH SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ YSTVPWTFGQGTKVEIKR
185	DVD840H	AB014VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMN WVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGS SHWYFDVWGQGTLVTVSS**ASTKGP**QVQLQESGPGL VKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKGL EWMGYISYSGNTRYQPSLKSRITISRDTSKNQFFL KLNSVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
186	DVD840L	AB014VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNW YQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

Example 2.33: Generation of EGFR (seq. 2) and DLL-4 DVD-Igs with Linker Set 1

Table 44

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
187	DVD339H	AB064VH	AB015VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGFTFTDNWISWVRQAPGKGLEWVGYISP NSGFTYYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCARDNFGGYFDYWGQGTLVTVSS
188	DVD339L	AB064VL	AB015VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCRASQ DVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRF SGSGSGTDFTLTISSLQPEDFATTYYCQQSYTGTV TFGQGTKVEIKR
189	DVD340H	AB015VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDNWIS WVRQAPGKGLEWVGYISPNSGFTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARDNFGGYF DYWGQGTLVTVSS**ASTKGP**QVQLQESGPGLVKPSQ TLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMGY ISYSGNTRYQPSLKSRITISRDTSKNQFFLKLNSV TAADTATYYCVTAGRGFPYWGQGTLVTVSS
190	DVD340L	AB015VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAW YQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATTYYCQQSYTGTVTFGQGTKV EIKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSS QDINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSR FSGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPW TFGGGTKLEIKR

Example 2.34: Generation of EGFR (seq. 2) and DLL-4 DVD-Igs with Linker Set 2

Table 45

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
191	DVD781H	AB064VH	AB015VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVESGGGL VQPGGSLRLSCAASGFTFTDNWISWVRQAPGKGLE WVGYISPNSGFTYYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCARDNFGGYFDYWGQGTLVTV SS
192	DVD781L	AB064VL	AB015VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLY SGVPSRFSGSGSGTDFTLTISSLQPEDFATTYYCQ QSYTGTVTFGQGTKVEIKR
193	DVD782H	AB015VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDNWIS WVRQAPGKGLEWVGYISPNSGFTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARDNFGGYF DYWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQESGP GLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGK GLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQF FLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVTV SS
194	DVD782L	AB015VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAW YQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATTYYCQQSYTGTVTFGQGTKV EIKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRV TITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNL DDGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCV QYAQFPWTFGGGTKLEIKR

Example 2.35: Generation of EGFR (seq. 2) and DLL-4 DVD-Igs with Linker Set 3

Table 46

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
195	DVD811H	AB064VH	AB015VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVESGGGL VQPGGSLRLSCAASGFTFTDNWISWVRQAPGKGLE WVGYISPNSGFTYYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCARDNFGGYFDYWGQGTLVTV SS
196	DVD811L	AB064VL	AB015VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCRASQ DVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYTGTV TFGQGTKVEIKR
197	DVD812H	AB015VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDNWIS WVRQAPGKGLEWVGYISPNSGFTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARDNFGGYF DYWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQESGP GLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGK GLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQF FLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVTV SS
198	DVD812L	AB015VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAW YQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATTYYCQQSYIGTVTFGQGTKV EIKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSS QDINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSR FSGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPW TFGGGTKLEIKR

Example 2.36: Generation of EGFR (seq. 2) and DLL-4 DVD-Igs with Linker Set 4

Table 47

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
199	DVD841H	AB064VH	AB015VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGFTFTDNWISWVRQAPGKGLEWVGYISP NSGFTYYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCARDNFGGYFDYWGQGTLVTVSS
200	DVD841L	AB064VL	AB015VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLY SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSYTGTVTFGQGTKVEIKR
201	DVD842H	AB015VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDNWIS WVRQAPGKGLEWVGYISPNSGFTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARDNFGGYF DYWGQGTLVTVSS**ASTKGP**QVQLQESGPGLVKPSQ TLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMGY ISYSGNTRYQPSLKSRITISRDTSKNQFFLKLNSV TAADTATYYCVTAGRGFPYWGQGTLVTVSS
202	DVD842L	AB015VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAW YQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYTGTVTFGQGTKV EIKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRV TITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNL DDGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCV QYAQFPWTFGGGTKLEIKR

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Example 2.37: Generation of EGFR (seq. 2) and PLGF DVD-Igs with Linker Set 1

Table 48

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
203	DVD341H	AB064VH	AB047VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**QVQLQQSGAELVKPGASV KISCKASGYTFTDYYINWVKLAPGQGLEWIGWIYP GSGNTKYNEKFKGKATLTIDTSSSTAYMQLSSLTS EDTAVYFCVRDSPFFDYWGQGTLLTVSS
204	DVD341L	AB064VL	AB047VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIVLTQSPDSLAVSLGERVTMNCKSSQ SLLNSGMRKSFLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISSVQAEDVAVYYCKQS YHLFTFGSGTKLEIKR
205	DVD342H	AB047VH	AB064VH	QVQLQQSGAELVKPGASVKISCKASGYTFTDYYIN WVKLAPGQGLEWIGWIYPGSGNTKYNEKFKGKATL TIDTSSSTAYMQLSSLTSEDTAVYFCVRDSPFFDY WGQGTLLTVSS**ASTKGP**QVQLQESGPGLVKPSQTL SLICTVSGYSISSDFAWNWIRQPPGKGLEWMGYIS YSGNTRYQPSLKSRITISRDTSKNQFFLKLNSVTA ADTATYYCVTAGRGFPYWGQGTLVTVSS
206	DVD342L	AB047VL	AB064VL	DIVLTQSPDSLAVSLGERVTMNCKSSQSLLNSGMR KSFLAWYQQKPGQSPKLLIYWASTRESGVPDRFTG SGSGTDFTLTISSVQAEDVAVYYCKQSYHLFTFGS GTKLEIKR**TVAAP**DIQMTQSPSSMSVSVGDRVTIT CHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDDG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQYA QFPWTFGGGTKLEIKR

Example 2.38: Generation of EGFR (seq. 2) and PLGF DVD-Igs with Linker Set 2

Table 49

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
207	DVD783H	AB064VH	AB047VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQQSGAEL VKPGASVKISCKASGYTFTDYYINWVKLAPGQGLE WIGWIYPGSGNTKYNEKFKGKATLTIDTSSSTAYM QLSSLTSEDTAVYFCVRDSPFFDYWGQGTLLTVSS
208	DVD783L	AB064VL	AB047VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIVLTQSPDSLAVSLGERVT MNCKSSQSLLNSGMRKSFLAWYQQKPGQSPKLLIY WASTRESGVPDRFTGSGSGTDFTLTISSVQAEDVA VYYCKQSYHLFTFGSGTKLEIKR
209	DVD784H	AB047VH	AB064Vh	QVQLQQSGAELVKPGASVKISCKASGYTFTDYYIN WVKLAPGQGLEWIGWIYPGSGNTKYNEKFKGKATL TIDTSSSTAYMQLSSLTSEDTAVYFCVRDSPFFDY WGQGTLLTVSS**ASTKGPSVFPLAP**QVQLQESGPGL VKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKGL EWMGYISYSGNTRYQPSLKSRITISRDTSKNQFFL KLNSVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
210	DVD784L	AB047VL	AB064VL	DIVLTQSPDSLAVSLGERVTMNCKSSQSLLNSGMR KSFLAWYQQKPGQSPKLLIYWASTRESGVPDRFTG SGSGTDFTLTISSVQAEDVAVYYCKQSYHLFTFGS GTKLEIKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSV GDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIYH GTNLDDGVPSRFSGSGSGTDYTLTISSLQPEDFAT YYCVQYAQFPWTFGGGTKLEIKR

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Example 2.39: Generation of EGFR (seq. 2) and PLGF DVD-Igs with Linker Set 3

Table 50

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
211	DVD813H	AB064VH	AB047VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQQSGAEL VKPGASVKISCKASGYTFTDYYINWVKLAPGQGLE WIGWIYPGSGNTKYNEKFKGKATLTIDTSSSTAYM QLSSLTSEDTAVYFCVRDSPFFDYWGQGTLLTVSS
212	DVD813L	AB064VL	AB047VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIVLTQSPDSLAVSLGERVTMNCKSSQ SLLNSGMRKSFLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISSVQAEDVAVYYCKQS YHLFTFGSGTKLEIKR
213	DVD814H	AB047VH	AB064VH	QVQLQQSGAELVKPGASVKISCKASGYTFTDYYIN WVKLAPGQGLEWIGWIYPGSGNTKYNEKFKGKATL TIDTSSSTAYMQLSSLTSEDTAVYFCVRDSPFFDY WGQGTLLTVSS**ASTKGPSVFPLAP**QVQLQESGPGL VKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKGL EWMGYISYSGNTRYQPSLKSRITISRDTSKNQFFL KLNSVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
214	DVD814L	AB047VL	AB064VL	DIVLTQSPDSLAVSLGERVTMNCKSSQSLLNSGMR KSFLAWYQQKPGQSPKLLIYWASTRESGVPDRFTG SGSGTDFTLTISSVQAEDVAVYYCKQSYHLFTFGS GTKLEIKR**TVAAP**DIQMTQSPSSMSVSVGDRVTIT CHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDDG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQYA QFPWTFGGGTKLEIKR

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Example 2.40: Generation of EGFR (seq. 2) and PLGF DVD-Igs with Linker Set 4

Table 51

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
215	DVD843H	AB064VH	AB047VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**QVQLQQSGAELVKPGASV KISCKASGYTFTDYYINWVKLAPGQGLEWIGWIYP GSGNTKYNEKFKGKATLTIDTSSSTAYMQLSSLTS EDTAVYFCVRDSPFFDYWGQGTLLTVSS
216	DVD843L	AB064VL	AB047VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIVLTQSPDSLAVSLGERVT MNCKSSQSLLNSGMRKSFLAWYQQKPGQSPKLLIY WASTRESGVPDRFTGSGSGTDFTLTISSVQAEDVA VYYCKQSYHLFTFGSGTKLEIKR
217	DVD844H	AB047VH	AB064VH	QVQLQQSGAELVKPGASVKISCKASGYTFTDYYIN WVKLAPGQGLEWIGWIYPGSGNTKYNEKFKGKATL TIDTSSSTAYMQLSSLTSEDTAVYFCVRDSPFFDY WGQGTLLTVSS**ASTKGP**QVQLQESGPGLVKPSQTL SLTCTVSGYSISSDFAWNWIRQPPGKGLEWMGYIS YSGNTRYQPSLKSRITISRDTSKNQFFLKLNSVTA ADTATYYCVTAGRGFPYWGQGTLVTVSS
218	DVD844L	AB047VL	AB064VL	DIVLTQSPDSLAVSLGERVTMNCKSSQSLLNSGMR KSFLAWYQQKPGQSPKLLIYWASTRESGVPDRFTG SGSGTDFTLTISSVQAEDVAVYYCKQSYHLFTFGS GTKLEIKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSV GDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIYH GTNLDDGVPSRFSGSGSGTDYTLTISSLQPEDFAT YYCVQYAQFPWTFGGGTKLEIKR

Example 2.41: Generation of EGFR (seq. 2) and ErbB3 (seq. 3) DVD-Igs with Linker Set 1

Table 52

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
219	DVD755H	AB064VH	AB067VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLLESGGGLVQPGGSL RLSCAASGFTFSHYVMAWVRQAPGKGLEWVSSISS SGGWTLYADSVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYCTRGLKMATIFDYWGQGTLVTVSS
220	DVD755L	AB064VL	AB067VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**QSALTQPASVSGSPGQSITISCTGTSS DVGSYNVVSWYQQHPGKAPKLIIYEVSQRPSGVSN RFSGSKSGNTASLTISGLQTEDEADYYCCSYAGSS IFVIFGGGTKVTVLG
221	DVD756H	AB067VH	AB064VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYVMA WVRQAPGKGLEWVSSISSSGGWTLYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCTRGLKMATI FDYWGQGTLVTVSS**ASTKGP**QVQLQESGPGLVKPS QTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMG YISYSGNTRYQPSLKSRITISRDTSKNQFFLKLNS VTAADTATYYCVTAGRGFPYWGQGTLVTVSS
222	DVD756L	AB067VL	AB064VL	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNVV SWYQQHPGKAPKLIIYEVSQRPSGVSNRFSGSKSG NTASLTISGLQTEDEADYYCCSYAGSSIFVIFGGG TKVTVLG**QPKAAP**DIQMTQSPSSMSVSVGDRVTIT CHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDDG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQYA QFPWTFGGGTKLEIKR

Example 2.42: Generation of EGFR (seq. 2) and ErbB3 (seq. 3) DVD-Igs with Linker Set 2

Table 53

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901 2345
223	DVD787H	AB064VH	AB067VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLLESGGGL VQPGGSLRLSCAASGFTFSHYVMAWVRQAPGKGLE WVSSISSSGGWTLYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCTRGLKMATIFDYWGQGTLVT VSS
224	DVD787L	AB064VL	AB067VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**QSALTQPASVSGSPGQSITI SCTGTSSDVGSYNVVSWYQQHPGKAPKLIIYEVSQ RPSGVSNRFSGSKSGNTASLTISGLQTEDEADYYC CSYAGSSIFVIFGGGTKVTVLG
225	DVD788H	AB067VH	AB064VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYVMA WVRQAPGKGLEWVSSISSSGGWTLYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCTRGLKMATI FDYWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQESG PGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPG KGLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQ FFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVT VSS
226	DVD788L	AB067VL	AB064VL	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNVV SWYQQHPGKAPKLIIYEVSQRPSGVSNRFSGSKSG NTASLTISGLQTEDEADYYCCSYAGSSIFVIFGGG TKVTVLG**QPKAAPSVTLFPP**DIQMTQSPSSMSVSV GDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIYH GTNLDDGVPSRFSGSGSGTDYTLTISSLQPEDFAT YYCVQYAQFPWTFGGGTKLEIKR

Example 2.43: Generation of EGFR (seq. 2) and ErbB3 (seq. 3) DVD-Igs with Linker Set 3

Table 54

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
227	DVD817H	AB064VH	AB067VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLLESGGGL VQPGGSLRLSCAASGFTFSHYVMAWVRQAPGKGLE WVSSISSSGGWTLYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCTRGLKMATIFDYWGQGTLVT VSS
228	DVD817L	AB064VL	AB067VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**QSALTQPASVSGSPGQSITISCTGTSS DVGSYNVVSWYQQHPGKAPKLIIYEVSQRPSGVSN RFSGSKSGNTASLTISGLQTEDEADYYCCSYAGSS IFVIFGGGTKVTVLG
229	DVD818H	AB067VH	AB064VH	EVQLLESGGGIVQPGGSLRLSCAASGFTFSHYVMA WVRQAPGKGLEWVSSISSSGGWTLYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCTRGLKMATI FDYWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQESG PGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPG KGLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQ FFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVT VSS
230	DVD818L	AB067VL	AB064VL	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNVV SWYQQHPGKAPKLIIYEVSQRPSGVSNRFSGSKSG NTASLTISGLQTEDEADYYCCSYAGSSIFVIFGGG TKVTVLG**QPKAAP**DIQMTQSPSSMSVSVGDRVTIT CHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDDG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQYA QFPWTFGGGTKLEIKR

Example 2.44: Generation of EGFR (seq. 2) and ErbB3 (seq. 3) DVD-Igs with Linker Set 4

Table 55

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
231	DVD847H	AB064VH	AB067VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLLESGGGLVQPGGSL RLSCAASGFTFSHYVMAWVRQAPGKGLEWVSSISS SGGWTLYADSVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYCTRGLKMATIFDYWGQGTLVTVSS
232	DVD847L	AB064VL	AB067VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**QSALTQPASVSGSPGQSITI SCTGTSSDVGSYNVVSWYQQHPGKAPKLIIYEVSQ RPSGVSNRFSGSKSGNTASLTISGLQTEDEADYYC CSYAGSSIFVIFGGGTKVTVLG
233	DVD848H	AB067VH	AB064VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYVMA WVRQAPGKGLEWVSSISSSGGWTLYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCTRGLKMATI FDYWGQGTLVTVSS**ASTKGP**QVQLQESGPGLVKPS QTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMG YISYSGNTRYQPSLKSRITISRDTSKNQFFLKLNS VTAADTATYYCVTAGRGFPYWGQGTLVTVSS
234	DVD848L	AB067VL	AB064VL	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNVV SWYQQHPGKAPKLIIYEVSQRPSGVSNRFSGSKSG NTASLTISGLQTEDEADYYCCSYAGSSIFVIFGGG TKVTVLG**QPKAAPSVTLFPP**DIQMTQSPSSMSVSV GDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIYH GTNLDDGVPSRFSGSGSGTDYTLTISSLQPEDFAT YYCVQYAQFPWTFGGGTKLEIKR

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Example 2.45: Generation of EGFR (seq. 2) and VEGF (seq. 2) DVD-Igs with Linker Set 1

Table 56

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
235	DVD757H	AB064VH	AB070VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGFTISDYWIHWVRQAPGKGLEWVAGITP AGGYTYYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCARFVFFLPYAMDYWGQGTLVTVSS
236	DVD757L	AB064VL	AB070VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCRASQ DVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYTTPPT FGQGTKVEIKR
237	DVD758H	AB070VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISDYWIH WVRQAPGKGLEWVAGITPAGGYTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARFVFFLPY AMDYWGQGTLVTVSS**ASTKGP**QVQLQESGPGLVKP SQTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWM GYISYSGNTRYQPSLKSRITISRDTSKNQFFLKLN SVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
238	DVD758L	AB070VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAW YQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYTTPPTFGQGTKVE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

Example 2.46: Generation of EGFR (seq. 2) and VEGF (seq. 2) DVD-Igs with Linker Set 2

Table 57

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
239	DVD789H	AB064VH	AB070VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVESGGGL VQPGGSLRLSCAASGFTISDYWIHWVRQAPGKGLE WVAGITPAGGYTYYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCARFVFFLPYAMDYWGQGTLV TVSS
240	DVD789L	AB064VL	AB070VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLY SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ SYTTPPTFGQGTKVEIKR
241	DVD790H	AB070VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISDYWIH WVRQAPGKGLEWVAGITPAGGYTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARFVFFLPY AMDYWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQES GPGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPP GKGLEWMGYISYSGNTRYQPSLKSRITISRDTSKN QFFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLV TVSS
242	DVD790L	AB070VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAW YQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYTTPPTFGQGTKVE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

Example 2.47: Generation of EGFR (seq. 2) and VEGF (seq. 2) DVD-Igs with Linker Set 3

Table 58

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
243	DVD819H	AB064VH	AB070VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVESGGGL VQPGGSLRLSCAASGFTISDYWIHWVRQAPGKGLE WVAGITPAGGYTYYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCARFVFFLPYAMDYWGQGTLV TVSS
244	DVD819L	AB064VL	AB070VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCRASQ DVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYTTPPT FGQGTKVEIKR
245	DVD820H	AB070VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISDYWIH WVRQAPGKGLEWVAGITPAGGYTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARFVFFLPY AMDYWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQES GPGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPP GKGLEWMGYISYSGNTRYQPSLKSRITISRDTSKN QFFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLV TVSS
246	DVD820L	AB070VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAW YQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYTTPPTFGQGTKVE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

Example 2.48: Generation of EGFR (seq. 2) and VEGF (seq. 2) DVD-Igs with Linker Set 4

Table 59

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
247	DVD849H	AB064VH	AB070VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGFTISDYWIHWVRQAPGKGLEWVAGITP AGGYTYYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCARFVFFLPYAMDYWGQGTLVTVSS
248	DVD849L	AB064VL	AB070VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLY SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ SYTTPPTFGQGTKVEIKR
249	DVD850H	AB070VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISDYWIH WVRQAPGKGLEWVAGITPAGGYTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARFVFFLPY AMDYWGQGTLVTVSS**ASTKGP**QVQLQESGPGLVKP SQTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWM GYISYSGNTRYQPSLKSRITISRDTSKNQFFLKLN SVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
250	DVD850L	AB070VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAW YQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYTTPPTFGQGTKVE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

Example 2.49: Generation of EGFR (seq. 2) and VEGF (seq. 3) DVD-Igs with Linker Set 1

Table 60

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
251	DVD759H	AB064VH	AB071VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGFTINASWIHWVRQAPGKGLEWVGAIYP YSGYTNYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCARWGHSTSPWAMDYWGQGTLVTVSS
252	DVD759L	AB064VL	AB071VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCRASQ VIRRSLAWYQQKPGKAPKLLIYAASNLASGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSNTSPLT FGQGTKVEIKR
253	DVD760H	AB071VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTINASWIH WVRQAPGKGLEWVGAIYPYSGYTNYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARWGHSTSP WAMDYWGQGTLVTVSS**ASTKGP**QVQLQESGPGLVK PSQTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEW MGYISYSGNTRYQPSLKSRITISRDTSKNQFFLKL NSVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
254	DVD760L	AB071VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQVIRRSLAW YQQKPGKAPKLLIYAASNLASGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNTSPLTFGQGTKVE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

Example 2.50: Generation of EGFR (seq. 2) and VEGF (seq. 3) DVD-Igs with Linker Set 2

Table 61

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
255	DVD791H	AB064VH	AB071VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVESGGGL VQPGGSLRLSCAASGFTINASWIHWVRQAPGKGLE WVGAIYPYSGYTNYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCARWGHSTSPWAMDYWGQGTL VTVSS
256	DVD791L	AB064VL	AB071VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCRASQVIRRSLAWYQQKPGKAPKLLIYAASNLA SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ SNTSPLTFGQGTKVEIKR
257	DVD792H	AB071VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTINASWIH WVRQAPGKGLEWVGAIYPYSGYTNYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARWGHSTSP WAMDYWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQE SGPGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQP PGKGLEWMGYISYSGNTRYQPSLKSRITISRDTSK NQFFLKLNSVTAADTATYYCVTAGRGFPYWGQGTL VTVSS
258	DVD792L	AB071VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQVIRRSLAW YQQKPGKAPKLLIYAASNLASGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNTSPLTFGQGTKVE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

Example 2.51: Generation of EGFR (seq. 2) and VEGF (seq. 3) DVD-Igs with Linker Set 3

Table 62

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
259	DVD821H	AB064VH	AB071VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVESGGGL VQPGGSLRLSCAASGFTINASWIHWVRQAPGKGLE WVGAIYPYSGYTNYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCARWGHSTSPWAMDYWGQGTL VTVSS
260	DVD821L	AB064VL	AB071VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCRASQ VIRRSLAWYQQKPGKAPKLLIYAASNLASGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSNTSPLT FGQGTKVEIKR
261	DVD822H	AB071VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTINASWIH WVRQAPGKGLEWVGAIYPYSGYTNYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARWGHSTSP WAMDYWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLQE SGPGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQP PGKGLEWMGYISYSGNTRYQPSLKSRITISRDTSK NQFFLKLNSVTAADTATYYCVTAGRGFPYWGQGTL VTVSS
262	DVD822L	AB071VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQVIRRSLAW YQQKPGKAPKLLIYAASNLASGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNTSPLTFGQGTKVE IKR**TVAAP**DIQMTQSPSSMSVSVGDRVTITCHSSQ DINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRF SGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWT FGGGTKLEIKR

Example 2.52: Generation of EGFR (seq. 2) and VEGF (seq. 3) DVD-Igs with Linker Set 4

Table 63

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
263	DVD851H	AB064VH	AB071VH	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAW NWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGFTINASWIHWVRQAPGKGLEWVGAIYP YSGYTNYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCARWGHSTSPWAMDYWGQGTLVTVSS
264	DVD851L	AB064VL	AB071VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGW LQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTD YTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLE IKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCRASQVIRRSLAWYQQKPGKAPKLLIYAASNLA SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ SNTSPLTFGQGTKVEIKR
265	DVD852H	AB071VH	AB064VH	EVQLVESGGGLVQPGGSLRLSCAASGFTINASWIH WVRQAPGKGLEWVGAIYPYSGYTNYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARWGHSTSP WAMDYWGQGTLVTVSS**ASTKGP**QVQLQESGPGLVK PSQTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEW MGYISYSGNTRYQPSLKSRITISRDTSKNQFFLKL NSVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
266	DVD852L	AB071VL	AB064VL	DIQMTQSPSSLSASVGDRVTITCRASQVIRRSLAW YQQKPGKAPKLLIYAASNLASGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNTSPLTFGQGTKVE IKR**TVAAPSVFIFPP**DIQMTQSPSSMSVSVGDRVT ITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLD DGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIKR

Example 2.53: Generation of EGFR (seq. 1) and RGMa DVD-Igs with Linker Set 1

Table 64

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
267	DVD713H	AB033VH	AB059VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE FAYWGQGTLVTVSA**ASTKGP**EVQLVESGGGLVQPG SSLKLSCVASGFTFSNYGMNWIRQAPKKGLEWIGM IYYDSSEKHYADSVKGRFTISRDNSKNTLYLEMNS LRSEDTAIYYCAKGTTPDYWGQGVMVTVSS
268	DVD713L	AB033VL	AB059VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAP**DVVLTQTPVSLSVTLGDQASMSCRSSQ SLEYSDGYTFLEWFLQKPGQSPQLLIYEVSNRFSG VPDRFIGSGSGTDFTLKISRVEPEDLGVYYCFQAT HDPLTFGSGTKLEIKR
269	DVD714H	AB059VH	AB033VH	EVQLVESGGGLVQPGSSLKLSCVASGFTFSNYGMN WIRQAPKKGLEWIGMIYYDSSEKHYADSVKGRFTI SRDNSKNTLYLEMNSLRSEDTAIYYCAKGTTPDYW GQGVMVTVSS**ASTKGP**QVQLKQSGPGLVQPSQSLS ITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSG GNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSND TAIYYCARALTYYDYEFAYWGQGTLVTVSA
270	DVD714L	AB059VL	AB033VL	DVVLTQTPVSLSVTLGDQASMSCRSSQSLEYSDGY TFLEWFLQKPGQSPQLLIYEVSNRFSGVPDRFIGS GSGTDFTLKISRVEPEDLGVYYCFQATHDPLTFGS GTKLEIKR**TVAAP**DILLTQSPVILSVSPGERVSFS CRASQSIGTNIHWYQQRTNGSPRLLIKYASESISG IPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNN NWPTTFGAGTKLELKR

Example 2.54: Generation of EGFR (seq. 1) and RGMa DVD-Igs with Linker Set 2

Table 65

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
271	DVD871H	AB033VH	AB059VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE FAYWGQGTLVTVSA**ASTKGPSVFPLAP**EVQLVESG GGLVQPGSSLKLSCVASGFTFSNYGMNWIRQAPKK GLEWIGMIYYDSSEKHYADSVKGRFTISRDNSKNT LYLEMNSLRSEDTAIYYCAKGTTPDYWGQGVMVTV SS
272	DVD871L	AB033VL	AB059VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAPSVFIFPP**DVVLTQTPVSLSVTLGDQAS MSCRSSQSLEYSDGYTFLEWFLQKPGQSPQLLIYE VSNRFSGVPDRFIGSGSGTDFTLKISRVEPEDLGV YYCFQATHDPLTFGSGTKLEIKR
273	DVD872H	AB059VH	AB033VH	EVQLVESGGGLVQPGSSLKLSCVASGFTFSNYGMN WIRQAPKKGLEWIGMIYYDSSEKHYADSVKGRFTI SRDNSKNTLYLEMNSLRSEDTAIYYCAKGTTPDYW GQGVMVTVSS**ASTKGPSVFPLAP**QVQLKQSGPGLV QPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEW LGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKM NSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTV SA
274	DVD872L	AB059VL	AB033VL	DVVLTQTPVSLSVTLGDQASMSCRSSQSLEYSDGY TFLEWFLQKPGQSPQLLIYEVSNRFSGVPDRFIGS GSGTDFTLKISRVEPEDLGVYYCFQATHDPLTFGS GTKLEIKR**TVAAPSVFIFPP**DILLTQSPVILSVSP GERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKY ASESISGIPSRFSGSGSGTDFTLSINSVESEDIAD YYCQQNNNWPTTFGAGTKLELKR

Example 2.55: Generation of EGFR (seq. 1) and RGMa DVD-Igs with Linker Set 3

Table 66

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
275	DVD877H	AB033VH	AB059VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE FAYWGQGTLVTVSA**ASTKGPSVFPLAP**EVQLVESG GGLVQPGSSLKLSCVASGFTFSNYGMNWIRQAPKK GLEWIGMIYYDSSEKHYADSVKGRFTISRDNSKNT LYLEMNSLRSEDTAIYYCAKGTTPDYWGQGVMVTV SS
276	DVD877L	AV033VL	AB059VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAP**DVVLTQTPVSLSVTLGDQASMSCRSSQ SLEYSDGYTFLEWFLQKPGQSPQLLIYEVSNRFSG VPDRFIGSGSGTDFTLKISRVEPEDLGVYYCFQAT HDPLTFGSGTKLE1KR
277	DVD878H	AB059VH	AB033VH	EVQLVESGGGLVQPGSSLKLSCVASGFTFSNYGMN WIRQAPKKGLEWIGMIYYDSSEKHYADSVKGRFTI SRDNSKNTLYLEMNSLRSEDTAIYYCAKGTTPDYW GQGVMVTVSS**ASTKGPSVFPLAP**QVQLKQSGPGLV QPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEW LGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKM NSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTV SA
278	DVD878L	AB059VL	AV033VL	DVVLTQTPVSLSVTLGDQASMSCRSSQSLEYSDGY TFLEWFLQKPGQSPQLL1YEVSNRFSGVPDRFIGS GSGTDFTLKISRVEPEDLGVYYCFQATHDPLTFGS GTKLEIKR**TVAAP**DILLTQSPVILSVSPGERVSFS CRASQSIGTNIHWYQQRTNGSPRLLIKYASESISG IPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNN NWPTTFGAGTKLELKR

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Example 2.56: Generation of EGFR (seq. 1) and RGMa DVD-Igs with Linker Set 4

Table 67

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
279	DVD883H	AB033VH	AB059VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN *KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE* FAYWGQGTLVTVSA**ASTKGP**EVQLVESGGGLVQPG SSLKLSCVASGFTFSNYGMNWIRQAPKKGLEWIGM IYYDSSEKHYADSVKGRFTISRDNSKNTLYLEMNS LRSEDTAIYYCAKGTTPDYWGQGVMVTVSS
280	DVD883L	AB033VL	AB059VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAPSVFIFPP**DVVLTQTPVSLSVTLGDQAS MSCRSSQSLEYSDGYTFLEWFLQKPGQSPQLLIYE VSNRFSGVPDRFIGSGSGTDFTLKISRVEPEDLGV YYCFQATHDPLTFGSGTKLEIKR
281	DVD884H	AB059VH	AB033VH	EVQLVESGGGLVQPGSSLKLSCVASGFTFSNYGMN WIRQAPKKGLEWIGMIYYDSSEKHYADSVKGRFTI SRDNSKNTLYLEMNSLRSEDTAIYYCAKGTTPDYW GQGVMVTVSS**ASTKGP**QVQLKQSGPGLVQPSQSLS ITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSG GNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSND TAIYYCARALTYYDYEFAYWGQGTLVTVSA
282	DVD884L	AB059VL	AB033VL	DVVLTQTPVSLSVTLGDQASMSCRSSQSLEYSDGY TFLEWFLQKPGQSPQLLIYEVSNRFSGVPDRFIGS GSGTDFTLKISRVEPEDLGVYYCFQATHDPLTFGS GTKLEIKR**TVAAPSVFIFPP**DILLTQSPVILSVSP GERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKY ASESISGIPSRFSGSGSGTDFTLSINSVESEDIAD YYCQQNNNWPTTFGAGTKLELKR

Example 2.57: Generation of EGFR (seq. 1) and Tetanus Toxoid DVD-Igs with Linker Set 1

Table 68

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
283	DVD763H	AB033VH	AB095VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN *KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE* FAYWGQGTLVTVSA**ASTKGP**EVQLLESGGDLVRPG GSLRLSCAASGFSFSRYGMSWVRQAPGKGLDWVAH ISASAGATYYADSVKGRFTISRDNSKNTLFLQMNN LRADDTAIYYCAKGGKQWLIPWFDPWGQGTLVTVS S
284	DVD763L	AB033VL	AB095VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAP**DIQMTQSPSSVSASVGDRVTIACRASQ DISDRLAWYQQKPGKVPKVLIYGASSLQSGVPSRF SGSGSGTDFTLTINSLQPEDFATYYCQQANSFPLT FGGGTKVEMKR
285	DVD764H	AB095VH	AB033VH	EVQLLESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGP**QVQLKQSGPGLVQ PSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWL GVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMN SLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVS A
286	DVD764L	AB095VL	AB033VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAP**DILLTQSPVILSVSPGERVSFSCRASQ SIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTT FGAGTKLELKR

Example 2.58: Generation of EGFR (seq. 1) and Tetanus Toxoid DVD-Igs with Linker Set 2

Table 69

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
287	DVD873H	AB033VH	AB095VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE FAYWGQGTLVTVSA**ASTKGPSVFPLAP**EVQLLESG GDLVRPGGSLRLSCAASGFSFSRYGMSWVRQAPGK GLDWVAHISASAGATYYADSVKGRFTISRDNSKNT LFLQMNNLRADDTAIYYCAKGGKQWLIPWFDPWGQ GTLVTVSS
288	DVD873L	AB033VL	AB095VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAPSVFIFPP**DIQMTQSPSSVSASVGDRVT IACRASQDISDRLAWYQQKPGKVPKVLIYGASSLQ SGVPSRFSGSGSGTDFTLTINSLQPEDFATYYCQQ ANSFPLTFGGGTKVEMKR
289	DVD874H	AB095VH	AB033VH	EVQLLESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLKQ SGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSP GKGLEWLGVIWSGGNTDYNTPFTSRLSINKDNSKS QVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQ GTLVTVSA
290	DVD874L	AB095VL	AB033VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAPSVFIFPP**DILLTQSPVILSVSPGERVS FSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESI SGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQ NNNWPTTFGAGTKLELKR

Example 2.59: Generation of EGFR (seq. 1) and Tetanus Toxoid DVD-Igs with Linker Set 3

Table 70

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
291	DVD879H	AB033VH	AB095VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE FAYWGQGTLVTVSA**ASTKGPSVFPLAP**EVQLLESG GDLVRPGGSLRLSCAASGFSFSRYGMSWVRQAPGK GLDWVAHISASAGATYYADSVKGRFTISRDNSKNT LFLQMNNLRADDTAIYYCAKGGKQWLIPWFDPWGQ GTLVTVSS
292	DVD879L	AV033VL	AB095VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAP**DIQMTQSPSSVSASVGDRVTIACRASQ DISDRLAWYQQKPGKVPKVLIYGASSLQSGVPSRF SGSGSGTDFTLTINSLQPEDFATYYCQQANSFPLT FGGGTKVEMKR
293	DVD880H	AB095VH	AB033VH	EVQLLESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGPSVFPLAP**QVQLKQ SGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSP GKGLEWLGVIWSGGNTDYNTPFTSRLSINKDNSKS QVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQ GTLVTVSA
294	DVD880L	AB095VL	AV033VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAP**DILLTQSPVILSVSPGERVSFSCRASQ SIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTT FGAGTKLELKR

Example 2.60: Generation of EGFR (seq. 1) and Tetanus Toxoid DVD-Igs with Linker Set 4

Table 71

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
295	DVD885H	AB033VH	AB095VH	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSIN KDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYE FAYWGQGTLVTVSA**ASTKGP**EVQLLESGGDLVRPG GSLRLSCAASGFSFSRYGMSWVRQAPGKGLDWVAH ISASAGATYYADSVKGRFTISRDNSKNTLFLQMNN LRADDTAIYYCAKGGKQWLIPWFDPWGQGTLVTVS S
296	DVD885L	AB033VL	AB095VL	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHW YQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTD FTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLE LKR**TVAAPSVFIFPP**DIQMTQSPSSVSASVGDRVT IACRASQDISDRLAWYQQKPGKVPKVLIYGASSLQ SGVPSRFSGSGSGTDFTLTINSLQPEDFATYYCQQ ANSFPLTFGGGTKVEMKR
297	DVD886H	AB095VH	AB033VH	EVQLLESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGP**QVQLKQSGPGLVQ PSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWL GVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMN SLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVS A
298	DVD886L	AB095VL	AB033VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAPSVFIFPP**DILLTQSPVILSVSPGERVS FSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESI SGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQ NNNWPTTFGAGTKLELKR

Example 2.61: Generation of VEGF (seq. 1) and Tetanus Toxoid DVD-Igs with Linker Set 1

Table 72

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
299	DVD869H	AB014VH	AB095VH	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMN WVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGS SHWYFDVWGQGTLVTVSS**ASTKGP**EVQLLESGGDL VRPGGSLRLSCAASGFSFSRYGMSWVRQAPGKGLD WVAHISASAGATYYADSVKGRFTISRDNSKNTLFL QMNNLRADDTAIYYCAKGGKQWLIPWFDPWGQGTL VTVSS
300	DVD869L	AB014VL	AB095VL	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNW YQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVE IKR**TVAAP**DIQMTQSPSSVSASVGDRVTIACRASQ DISDRLAWYQQKPGKVPKVLIYGASSLQSGVPSRF SGSGSGTDFTLTINSLQPEDFATYYCQQANSFPLT FGGGTKVEMKR
301	DVD870H	AB095VH	AB014VH	EVQLLESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQ PGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWV GWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQM NSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL VTVSS
302	DVD870L	AB095VL	AB014VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCSASQ DISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWT FGQGTKVEIKR

Example 2.62: Generation of VEGF (seq. 1) and Tetanus Toxoid DVD-Igs with Linker Set 2

Table 73

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
303	DVD875H	AB014VH	AB095VH	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMN WVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGS SHWYFDVWGQGTLVTVSS**ASTKGPSVFPLAP**EVQL LESGGDLVRPGGSLRLSCAASGFSFSRYGMSWVRQ APGKGLDWVAHISASAGATYYADSVKGRFTISRDN SKNTLFLQMNNLRADDTAIYYCAKGGKQWLIPWFD PWGQGTLVTVSS
304	DVD875L	AB014VL	AB095VL	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNW YQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVE IKR**TVAAPSVFIFPP**DIQMTQSPSSVSASVGDRVT ITCRASQDISDRLAWYQQKPGKVPKVLIYGASSLQ SGVPSRFSGSGSGTDFTLTINSLQPEDFATYYCQQ ANSFPLTFGGGTKVEMKR
305	DVD876H	AB095VH	AB014VH	EVQLLESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVE SGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAP GKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSK STAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFD VWGQGTLVTVSS
306	DVD876L	AB095VL	AB014VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLH SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ YSTVPWTFGQGTKVEIKR

Example 2.63: Generation of VEGF (seq. 1) and Tetanus Toxoid DVD-Igs with Linker Set 3

Table 74

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
307	DVD881H	AB014VH	AB095VH	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMN WVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGS SHWYFDVWGQGTLVTVSS**ASTKGPSVFPLAP**EVQL LESGGDLVRPGGSLRLSCAASGFSFSRYGMSWVRQ APGKGLDWVAHISASAGATYYADSVKGRFTISRDN SKNTLFLQMNNLRADDTAIYYCAKGGKQWLIPWFD PWGQGTLVTVSS
308	DVD881L	AB014VL	AB095VL	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNW YQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVE IKR**TVAAP**DIQMTQSPSSVSASVGDRVTIACRASQ DISDRLAWYQQKPGKVPKVLIYGASSLQSGVPSRF SGSGSGTDFTLTINSLQPEDFATYYCQQANSFPLT FGGGTKVEMKR
309	DVD882H	AB095VH	AB014VH	EVQLIESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGPSVFPLAP**EVQLVE SGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAP GKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSK STAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFD VWGQGTLVTVSS
310	DVD882L	AB095VL	AB014VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAP**DIQMTQSPSSLSASVGDRVTITCSASQ DISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWT FGQGTKVEIKR

Example 2.64: Generation of VEGF (seq. 1) and Tetanus Toxoid DVD-Igs with Linker Set 4

Table 75

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
311	DVD887H	AB014VH	AB095VH	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMN WVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGS SHWYFDVWGQGTLVTVSS**ASTKGP**EVQLLESGGDL VRPGGSLRLSCAASGFSFSRYGMSWVRQAPGKGLD WVAHISASAGATYYADSVKGRFTISRDNSKNTLFL QMNNLRADDTAIYYCAKGGKQWLIPWFDPWGQGTL VTVSS
312	DVD887L	AB014VL	AB095VL	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNW YQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVE IKR**TVAAPSVFIFPP**DIQMTQSPSSVSASVGDRVT IACRASQDISDRLAWYQQKPGKVPKVLIYGASSLQ SGVPSRFSGSGSGTDFTLTINSLQPEDFATYYCQQ ANSFPLTFGGGTKVEMKR
313	DVD888H	AB095VH	AB014VH	EVQLLESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQ PGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWV GWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQM NSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL VTVSS
314	DVD888L	AB095VL	AB014VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAPSVFIFPP**DIQMTQSPSSLSASVGDRVT ITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLH SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ YSTVPWTFGQGTKVEIKR

Example 2.65: Generation of Tetanus Toxoid and Tetanus Toxoid DVD-Igs with Linker Set 1

Table 76

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
315	DVD889H	AB095VH	AB095VH	EVQLLESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGP**EVQLLESGGDLVR PGGSLRLSCAASGFSFSRYGMSWVRQAPGKGLDWV AHISASAGATYYADSVKGRFTISRDNSKNTLFLQM NNLRADDTAIYYCAKGGKQWLIPWFDPWGQGTLVT VSS
316	DVD889L	AB095VL	AB095VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAP**DIQMTQSPSSVSASVGDRVTIACRASQ DISDRLAWYQQKPGKVPKVLIYGASSLQSGVPSRF SGSGSGTDFTLTINSLQPEDFATYYCQQANSFPLT FGGGTKVEMKR
317	DVD890H	AB095VH	AB095VH	EVQLLESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGPSVFPLAP**EVQLLE SGGDLVRPGGSLRLSCAASGFSFSRYGMSWVRQAP GKGLDWVAHISASAGATYYADSVKGRFTISRDNSK NTLFLQMNNLRADDTAIYYCAKGGKQWLIPWFDPW GQGTLVTVSS
318	DVD890L	AB095VL	AB095VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAPSVFIFPP**DIQMTQSPSSVSASVGDRVT IACRASQDISDRLAWYQQKPGKVPKVLIYGASSLQ SGVPSRFSGSGSGTDFTLTINSLQPEDFATYYCQQ ANSFPLTFGGGTKVEMKR

Example 2.66: Generation of Tetanus Toxoid and Tetanus Toxoid DVD-Igs with Linker Set 2

Table 77

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 1234567890123456789012345678901234 5
319	DVD891H	AB095VH	AB095VH	EVQLLESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGPSVFPLAP**EVQLLE SGGDLVRPGGSLRLSCAASGFSFSRYGMSWVRQAP GKGLDWVAHISASAGATYYADSVKGRFTISRDNSK NTLFLQMNNLRADDTAIYYCAKGGKQWLIPWFDPW GQGTLVTVSS
320	DVD891L	AB095VL	AB095VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAP**DIQMTQSPSSVSASVGDRVTIACRASQ DISDRLAWYQQKPGKVPKVLIYGASSLQSGVPSRF SGSGSGTDFTLTINSLQPEDFATYYCQQANSFPLT FGGGTKVEMKR
321	DVD892H	AB095VH	AB095VH	EVQLLESGGDLVRPGGSLRLSCAASGFSFSRYGMS WVRQAPGKGLDWVAHISASAGATYYADSVKGRFTI SRDNSKNTLFLQMNNLRADDTAIYYCAKGGKQWLI PWFDPWGQGTLVTVSS**ASTKGP**EVQLLESGGDLVR PGGSLRLSCAASGFSFSRYGMSWVRQAPGKGLDWV AHISASAGATYYADSVKGRFTISRDNSKNTLFLQM NNLRADDTAIYYCAKGGKQWLIPWFDPWGQGTLVT VSS
322	DVD892L	AB095VL	AB095VL	DIQMTQSPSSVSASVGDRVTIACRASQDISDRLAW YQQKPGKVPKVLIYGASSLQSGVPSRFSGSGSGTD FTLTINSLQPEDFATYYCQQANSFPLTFGGGTKVE MKR**TVAAPSVFIFPP**DIQMTQSPSSVSASVGDRVT IACRASQDISDRLAWYQQKPGKVPKVLIYGASSLQ SGVPSRFSGSGSGTDFTLTINSLQPEDFATYYCQQ ANSFPLTFGGGTKVEMKR

The present invention incorporates by reference in their entirety techniques well known in the field of molecular biology and drug delivery. These techniques include, but are not limited to, techniques described in the following publications:

Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley &Sons, NY (1993);
Ausubel, F.M. et al. eds., Short Protocols In Molecular Biology (4th Ed. 1999) John Wiley & Sons, NY. (ISBN 0-471-32938-X).
Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984);
Giege, R. and Ducruix, A. Barrett, Crystallization of Nucleic Acids and Proteins, a Practical Approach, 2nd ea., pp. 20 1-16, Oxford University Press, New York, New York, (1999);
Goodson, in Medical Applications of Controlled Release, vol. 2, pp. 115-138 (1984);
Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981;
Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988);
Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991);
Kabat, E.A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242;
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Incorporation by Reference

The contents of all cited references (including literature references, patents, patent applications, and websites) that maybe cited throughout this application are hereby expressly incorporated by reference in their entirety, as are the references cited therein. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology and cell biology, which are well known in the art.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting of the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

CLAIMS

1. A binding protein comprising first and second polypeptide chains, each independently comprising VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first variable domain;

VD2 is a second variable domain;

C is constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 is an Fc region;

n is 0 or 1,

wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site and the VD2 domains on the first and second polypeptide chains form a second functional target binding site, and wherein the binding protein is capable of binding:

a. EGFR and CD3, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,

three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or

three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328,

and

the binding protein is capable of binding to EGFR with an EC50 of at most 157.62 nM as measured by direct binding ELISA and/or with an IC50 of at most 16 pM as measured by rCTL activity, and

the variable domains that form a functional target binding site for CD3 comprise three CDRs from SEQ ID NO: 27 and three CDRs from SEQ ID NO: 28;

b. EGFR and IGF1R, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,

three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or

three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328,

and the binding protein is capable of binding to EGFR with an EC50 of at most 286.41 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for IGFR1 comprise three CDRs from SEQ ID NO: 31 and three CDRs from SEQ ID NO: 32 and the binding protein is capable of binding to IGF1R with an EC50 of at most 78.58 nM as measured by direct binding ELISA;

c. EGFR and RON, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,
three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,
three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or
three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328,
and

the binding protein is capable of binding to EGFR with an EC50 of at most 29.93 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for RON comprise three CDRs from SEQ ID NO: 29 and three CDRs from SEQ ID NO: 30 and the binding protein is capable of binding to RON with an EC50 of at most 24.99 nM as measured by direct binding ELISA;

d. EGFR and HGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,
three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,
three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or
three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328,
and

the binding protein is capable of binding to EGFR with an EC50 of at most 332.05 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for HGF comprise three CDRs from SEQ ID NO: 33 and three CDRs from SEQ ID NO: 34 and the binding protein is capable of binding to HGF with an EC50 of at most 159.04 nM as measured by direct binding ELISA;

e. EGFR and VEGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,
three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328, and

the binding protein is capable of binding to EGFR with an EC50 of at most 561.59 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for VEGF comprise three CDRs from SEQ ID NO: 35 and three CDRs from SEQ ID NO: 36, three CDRs from SEQ ID NO: 53 and three CDRs from SEQ ID NO: 54, or three CDRs from SEQ ID NO: 55 and three CDRs from SEQ ID NO: 56,

and the binding protein is capable of binding to VEGF with an EC50 of at most 528.26 nM as measured by direct binding ELISA;

f. EGFR and Erb33, wherein:

the variable domains that form a functional target binding site for EGFR comprise three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50, three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324, three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328, and

the binding protein is capable of binding to EGFR with an EC50 of at most 726.39 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for Erb33 comprise three CDRs from SEQ ID NO: 45 and three CDRs from SEQ ID NO: 46, three CDRs from SEQ ID NO: 47 and three CDRs from SEQ ID NO: 48, or three CDRs from SEQ ID NO: 51 and three CDRs from SEQ ID NO: 52, and

the binding protein is capable of binding to Erb33 with an EC50 of at most 485.26 nM as measured by direct binding ELISA;

g. EGFR and DLL-4, wherein:

the variable domains that form a functional target binding site for EGFR comprise three CDRs from SEQ ID NO: 39 and three CDRs from SEQ ID NO: 40, three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50, three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324, three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328, and

the binding protein is capable of binding to EGFR with an EC50 of at most 189.14 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for DLL-4 comprise three CDRs from SEQ ID NO: 37 and three CDRs from SEQ ID NO: 38 and the binding protein is capable of binding to DLL-4 with an EC50 of at most 4.01 nM as measured by direct binding ELISA;

h. EGFR and PLGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

three CDRs from SEQ ID NO: 39 and three CDRs from SEQ ID NO: 40,

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,

three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324,

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, or

three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328, and

the binding protein is capable of binding to EGFR with an EC50 of at most 119.88 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for PLGF comprise three CDRs from SEQ ID NO: 41 and three CDRs from SEQ ID NO: 42 and the binding protein is capable of binding to PLGF with an EC50 of at most 0.74 nM as measured by direct binding ELISA;

or

i. EGFR and EGFR, wherein:

the variable domains that form functional target binding sites for EGFR each independently comprise

three CDRs from SEQ ID NO: 39 and three CDRs from SEQ ID NO: 40,

three CDRs from SEQ ID NO: 49 and three CDRs from SEQ ID NO: 50,

three CDRs from SEQ ID NO: 323 and three CDRs from SEQ ID NO: 324

three CDRs from SEQ ID NO: 325 and three CDRs from SEQ ID NO: 326, and/or

three CDRs from SEQ ID NO: 327 and three CDRs from SEQ ID NO: 328, and

the binding protein is capable of binding to EGFR with an EC50 of at most 6.83 nM as measured by direct binding ELISA and/or with a KD of at most 4.20E-08 M as measured by surface plasmon resonance.

2. A binding protein comprising first and second polypeptide chains, each independently comprising VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first variable domain;

VD2 is a second variable domain;

C is constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 is an Fc region;

n is 0 or 1,

wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site and the VD2 domains on the first and second polypeptide chains form a second functional target binding site, and wherein the binding protein is capable of binding:

a. EGFR and CD3, wherein:
the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NOs: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 6.83 nM as measured by direct binding ELISA and/or with a KD of at most 4.20E-08 M as measured by surface plasmon resonance, and
the variable domains that form a functional target binding site for CD3 comprise SEQ ID NO: 27 and/or SEQ ID NO: 28;

b. EGFR and IGF1R, wherein:
the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NOs: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 286.41 nM as measured by direct binding ELISA, and
the variable domains that form a functional target binding site for IGFR1 comprise SEQ ID NO: 31 and/or SEQ ID NO: 32 and the binding protein is capable of binding to IGF1R with an EC50 of at most 78.58 nM as measured by direct binding ELISA;

c. EGFR and RON, wherein:
the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NOs: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 29.93 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for RON comprise SEQ ID NO: 29 and/or SEQ ID NO: 30 and the binding protein is capable of binding to RON with an EC50 of at most 24.99 nM as measured by direct binding ELISA;

d. EGFR and HGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NOs: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 332.05 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for HGF comprise SEQ ID NO: 33 and/or SEQ ID NO: 34 and the binding protein is capable of binding to HGF with an EC50 of at most 159.04 nM as measured by direct binding ELISA;

e. EGFR and VEGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NO: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 561.59 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for VEGF comprise a sequence selected from a group consisting of SEQ ID NO: 35, 36, and 53-56 and the binding protein is capable of binding to VEGF with an EC50 of at most 528.26 nM as measured by direct binding ELISA;

f. EGFR and Erb33, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NO: 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 726.39 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for Erb33 comprise a sequence selected from a group consisting of SEQ ID NO: 45-48 and 51-52 and the binding protein is capable of binding to Erb33 with an EC50 of at most 485.26 nM as measured by direct binding ELISA;

g. EGFR and DLL-4, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NO: 39, 40, 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 189.14 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for DLL-4 comprise SEQ ID NO: 37 and/or SEQ ID NO: 38 and the binding protein is capable of binding to DLL-4 with an EC50 of at most 4.01 nM as measured by direct binding ELISA;

h. EGFR and PLGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise a sequence selected from a group consisting of SEQ ID NO: 39, 40, 49, 50, and 323-28 and the binding protein is capable of binding to EGFR with an EC50 of at most 119.88 nM as measured by direct binding ELISA, and

the variable domains that form a functional target binding site for PLGF comprise SEQ ID NO: 41 and/or SEQ ID NO: 42 and the binding protein is capable of binding to PLGF with an EC50 of at most 0.74 nM as measured by direct binding ELISA;

or

i. EGFR and EGFR, wherein:

the variable domains that form functional target binding sites for EGFR each independently comprise a sequence selected from a group consisting of SEQ ID NO: 39, 40, 49, 50, and 323-28 and is capable of binding to EGFR with an EC50 of at most 6.83 nM as measured by direct binding ELISA and/or with a KD of at most 4.20x10-08 M as measured by surface plasmon resonance.

3. The binding protein according to any one of claim 1 or claim 2, comprising first and second polypeptide chains, wherein

a. the first polypeptide chain comprises a first VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first heavy chain variable domain;

VD2 is a second heavy chain variable domain;

C is a heavy chain constant domain;

X1 is a first linker with the proviso that it is not CH1;

X2 is an Fc region;

n is 0 or 1; and

b. the second polypeptide chain comprises a second VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first light chain variable domain;

VD2 is a second light chain variable domain;

C is a light chain constant domain;

X1 is a second linker with the proviso that it is not CH1;

X2 does not comprise an Fc region;

n is 0 or 1.

4. A binding protein according to any one of claims 1-3, wherein the binding protein comprises variable domains that form functional binding sites for

a. EGFR and CD3, wherein the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for CD3 comprise SEQ ID NO: 27 and SEQ ID NO: 28;

b. EGFR and IGF1R, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for IGFR1 comprise SEQ ID NO: 31 and SEQ ID NO: 32;

c. EGFR and RON, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for RON comprise SEQ ID NO: 29 and SEQ ID NO: 30;

d. EGFR and HGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for HGF comprise SEQ ID NO: 33 and SEQ ID NO: 34;

e. EGFR and VEGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for VEGF comprise

SEQ ID NO: 35 and SEQ ID NO: 36,

SEQ ID NO: 53 and SEQ ID NO: 54, or

SEQ ID NO: 55 and SEQ ID NO: 56;

f. EGFR and Erb33, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for Erb33 comprise

SEQ ID NO: 45 and SEQ ID NO: 46,

SEQ ID NO: 47 and SEQ ID NO: 48, or

SEQ ID NO: 51 and SEQ ID NO: 52;

g. EGFR and DLL-4, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 39 and SEQ ID NO: 40,

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for DLL-4 comprise SEQ ID NO: 37 and SEQ ID NO: 38;

h. EGFR and PLGF, wherein:

the variable domains that form a functional target binding site for EGFR comprise

SEQ ID NO: 39 and SEQ ID NO: 40,

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328, and

the variable domains that form a functional target binding site for PLGF comprise SEQ ID NO: 41 and SEQ ID NO: 42;

or

i. EGFR and EGFR, wherein:

the variable domains that form functional target binding sites for EGFR each independently comprise

SEQ ID NO: 39 and SEQ ID NO: 40,

SEQ ID NO: 49 and SEQ ID NO: 50,

SEQ ID NO: 323 and SEQ ID NO: 324,

SEQ ID NO: 325 and SEQ ID NO: 326, or

SEQ ID NO: 327 and SEQ ID NO: 328.

5. The binding protein according to any one of claims 1-4, wherein the binding protein is capable of binding to

a. EGFR and CD3, and comprises

DVD331 (comprising SEQ ID NO: 123 and SEQ ID NO: 124),

DVD332 (comprising SEQ ID NO: 125 and SEQ ID NO: 126),

DVD773 (comprising SEQ ID NO: 127 and SEQ ID NO: 128),

DVD774 (comprising SEQ ID NO: 129 and SEQ ID NO: 130),

DVD803 (comprising SEQ ID NO: 131 and SEQ ID NO: 132),

DVD804 (comprising SEQ ID NO: 133 and SEQ ID NO: 134),

DVD833 (comprising SEQ ID NO: 135 and SEQ ID NO: 136), or

DVD834 (comprising SEQ ID NO: 137 and SEQ ID NO: 138);

b. EGFR and IGF1R, and comprises

DVD333 (comprising SEQ ID NO: 139 and SEQ ID NO: 140),

DVD334 (comprising SEQ ID NO: 141 and SEQ ID NO: 142),

DVD775 (comprising SEQ ID NO: 143 and SEQ ID NO: 144),

DVD776 (comprising SEQ ID NO: 145 and SEQ ID NO: 146),

DVD805 (comprising SEQ ID NO: 147 and SEQ ID NO: 148),

DVD806 (comprising SEQ ID NO: 149 and SEQ ID NO: 150),

DVD835 (comprising SEQ ID NO: 151 and SEQ ID NO: 152), or

DVD836 (comprising SEQ ID NO: 153 and SEQ ID NO: 154);

c. EGFR and RON, and comprises

DVD325 (comprising SEQ ID NO: 75 and SEQ ID NO: 76),

DVD326 (comprising SEQ ID NO: 77 and SEQ ID NO: 78),

DVD767 (comprising SEQ ID NO: 79 and SEQ ID NO: 80),

DVD768 (comprising SEQ ID NO: 81 and SEQ ID NO: 82),

DVD797 (comprising SEQ ID NO: 83 and SEQ ID NO: 84),

DVD798 (comprising SEQ ID NO: 85 and SEQ ID NO: 86),

DVD827 (comprising SEQ ID NO: 87 and SEQ ID NO: 88), or

DVD828 (comprising SEQ ID NO: 89 and SEQ ID NO: 90);

d. EGFR and HGF, and comprises

DVD335 (comprising SEQ ID NO: 155 and SEQ ID NO: 156),

DVD336 (comprising SEQ ID NO: 157 and SEQ ID NO: 158),

DVD777 (comprising SEQ ID NO: 159 and SEQ ID NO: 160),

DVD778 (comprising SEQ ID NO: 161 and SEQ ID NO: 162),

DVD807 (comprising SEQ ID NO: 163 and SEQ ID NO: 164),

DVD808 (comprising SEQ ID NO: 165 and SEQ ID NO: 166),

DVD837 (comprising SEQ ID NO: 167 and SEQ ID NO: 168), or

DVD838 (comprising SEQ ID NO: 169 and SEQ ID NO: 170);

e. EGFR and VEGF, and comprises

DVD337 (comprising SEQ ID NO: 171 and SEQ ID NO: 172),

DVD338 (comprising SEQ ID NO: 173 and SEQ ID NO: 174),

DVD779 (comprising SEQ ID NO: 175 and SEQ ID NO: 176),

DVD780 (comprising SEQ ID NO: 177 and SEQ ID NO: 178),

DVD809 (comprising SEQ ID NO: 179 and SEQ ID NO: 180),

DVD810 (comprising SEQ ID NO: 181 and SEQ ID NO: 182),

DVD839 (comprising SEQ ID NO: 183 and SEQ ID NO: 184),

DVD840 (comprising SEQ ID NO: 185 and SEQ ID NO: 186),

DVD757 (comprising SEQ ID NO: 235 and SEQ ID NO: 236),

DVD758 (comprising SEQ ID NO: 237 and SEQ ID NO: 238),

DVD789 (comprising SEQ ID NO: 239 and SEQ ID NO: 240),

DVD790 (comprising SEQ ID NO: 241 and SEQ ID NO: 242),

DVD819 (comprising SEQ ID NO: 243 and SEQ ID NO: 244),
DVD820 (comprising SEQ ID NO: 245 and SEQ ID NO: 246),
DVD849 (comprising SEQ ID NO: 247 and SEQ ID NO: 248),
DVD850 (comprising SEQ ID NO: 249 and SEQ ID NO: 250),
DVD759 (comprising SEQ ID NO: 251 and SEQ ID NO: 252),
DVD760 (comprising SEQ ID NO: 253 and SEQ ID NO: 254),
DVD791 (comprising SEQ ID NO: 255 and SEQ ID NO: 256),
DVD792 (comprising SEQ ID NO: 257 and SEQ ID NO: 258),
DVD821 (comprising SEQ ID NO: 259 and SEQ ID NO: 260),
DVD822 (comprising SEQ ID NO: 261 and SEQ ID NO: 262),
DVD851 (comprising SEQ ID NO: 263 and SEQ ID NO: 264), or
DVD852 (comprising SEQ ID NO: 265 and SEQ ID NO: 266);

f. EGFR and Erb33, and comprises

DVD327 (comprising SEQ ID NO: 91 and SEQ ID NO: 92),
DVD328 (comprising SEQ ID NO: 93 and SEQ ID NO: 94),
DVD769 (comprising SEQ ID NO: 95 and SEQ ID NO: 96),
DVD770 (comprising SEQ ID NO: 97 and SEQ ID NO: 98),
DVD799 (comprising SEQ ID NO: 99 and SEQ ID NO: 100),
DVD800 (comprising SEQ ID NO: 101 and SEQ ID NO: 102),
DVD829 (comprising SEQ ID NO: 103 and SEQ ID NO: 104),
DVD830 (comprising SEQ ID NO: 105 and SEQ ID NO: 106),
DVD329 (comprising SEQ ID NO: 107 and SEQ ID NO: 108),
DVD330 (comprising SEQ ID NO: 109 and SEQ ID NO: 110),
DVD771 (comprising SEQ ID NO: 111 and SEQ ID NO: 112),
DVD772 (comprising SEQ ID NO: 113 and SEQ ID NO: 114),
DVD801 (comprising SEQ ID NO: 115 and SEQ ID NO: 116),
DVD802 (comprising SEQ ID NO: 117 and SEQ ID NO: 118),
DVD831 (comprising SEQ ID NO: 119 and SEQ ID NO: 120),
DVD832 (comprising SEQ ID NO: 121 and SEQ ID NO: 122),
DVD755 (comprising SEQ ID NO: 219 and SEQ ID NO: 220),
DVD756 (comprising SEQ ID NO: 221 and SEQ ID NO: 222),
DVD787 (comprising SEQ ID NO: 223 and SEQ ID NO: 224),
DVD788 (comprising SEQ ID NO: 225 and SEQ ID NO: 226),
DVD817 (comprising SEQ ID NO: 227 and SEQ ID NO: 228),

DVD818 (comprising SEQ ID NO: 229 and SEQ ID NO: 230),

DVD847 (comprising SEQ ID NO: 231 and SEQ ID NO: 232), or

DVD848 (comprising SEQ ID NO: 233 and SEQ ID NO: 234);

g. EGFR and DLL-4, and comprises

DVD339 (comprising SEQ ID NO: 187 and SEQ ID NO: 188),

DVD340 (comprising SEQ ID NO: 189 and SEQ ID NO: 190),

DVD781 (comprising SEQ ID NO: 191 and SEQ ID NO: 192),

DVD782 (comprising SEQ ID NO: 193 and SEQ ID NO: 194),

DVD811 (comprising SEQ ID NO: 195 and SEQ ID NO: 196),

DVD812 (comprising SEQ ID NO: 197 and SEQ ID NO: 198),

DVD841 (comprising SEQ ID NO: 199 and SEQ ID NO: 200), or

DVD842 (comprising SEQ ID NO: 201 and SEQ ID NO: 202);

h. EGFR and PLGF, and comprises

DVD341 (comprising SEQ ID NO: 203 and SEQ ID NO: 204),

DVD342 (comprising SEQ ID NO: 205 and SEQ ID NO: 206),

DVD783 (comprising SEQ ID NO: 207 and SEQ ID NO: 208),

DVD784 (comprising SEQ ID NO: 209 and SEQ ID NO: 210),

DVD813 (comprising SEQ ID NO: 211 and SEQ ID NO: 212),

DVD814 (comprising SEQ ID NO: 213 and SEQ ID NO: 214),

DVD843 (comprising SEQ ID NO: 215 and SEQ ID NO: 216), or

DVD844 (comprising SEQ ID NO: 217 and SEQ ID NO: 218);

or

i. EGFR and EGFR, and comprises

DVD321 (comprising SEQ ID NO: 59 and SEQ ID NO: 60),

DVD322 (comprising SEQ ID NO: 61 and SEQ ID NO: 62),

DVD765 (comprising SEQ ID NO: 63 and SEQ ID NO: 64),

DVD766 (comprising SEQ ID NO: 65 and SEQ ID NO: 66),

DVD795 (comprising SEQ ID NO: 67 and SEQ ID NO: 68),

DVD796 (comprising SEQ ID NO: 69 and SEQ ID NO: 70),

DVD825 (comprising SEQ ID NO: 71 and SEQ ID NO: 72), or

DVD826 (comprising SEQ ID NO: 73 and SEQ ID NO: 74).

6. The binding protein according to any one of claims 1-5 comprising two first polypeptide chains and two second polypeptide chains, wherein the binding protein comprises four functional target binding sites.

7. The binding protein according to any one of claims 1-6, wherein

a. X1 is not CL;

b. X1 is at least one of SEQ ID NO: 1-26 or a G/S based sequence;

c. the binding protein is a crystallized binding protein;

d. the Fc region is a variant sequence Fc region; and/or

e. the Fc region is from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, or IgD.

8. A binding protein conjugate comprising a binding protein according to any one of claims 1-7, the binding protein conjugate further comprising an immunoadhesion molecule, an imaging agent, a therapeutic agent, or a cytotoxic agent.

9. The binding protein conjugate according to claim 8, wherein the imaging agent is a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, or biotin.

10. The binding protein conjugate according to claim 9, wherein the radiolabel is 3H, 14C, 35S, 90Y, 99Tc, 111In, 125I, 131I, 177Lu, 166Ho, or 153Sm.

11. The binding protein conjugate according to claim 8, wherein the therapeutic or cytotoxic agent is an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, a toxin, or an apoptotic agent.

12. A pharmaceutical composition comprising the binding protein of any one of claims 1-7 or the binding protein conjugate of claims 8-11, and a pharmaceutically acceptable carrier, optionally comprising at least one additional therapeutic agent selected from the group consisting of an imaging agent, a cytotoxic agent, an angiogenesis inhibitor, a kinase inhibitor, a co-stimulation molecule blocker, an adhesion molecule blocker, an anti-cytokine antibody or functional fragment thereof, methotrexate, cyclosporin, rapamycin, FK506, a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NTHE), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteriod, an anabolic steroid,

an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, and a cytokine antagonist.

13. Use of the binding protein according to claims 1-7 or the binding protein conjugate according to any one of claims 8-11 in the manufacture of a medicament for treating a disease or disorder.

14. The use of claim 13, wherein the disorder is arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea, microscopic vasculitis ofthe kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthopathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthopathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung

disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjogren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-l autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, psoriasis type I, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasulitis of the kidneys, lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjorgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatatis, idiosyncratic liver disease, Drug-Induced hepatitis, Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (e.g., depression and schizophrenia), Th2 Type and Thl Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), Abetalipoprotemia, Acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-l-

antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior hom cell degeneration, anti cd3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aortic and peripheral aneuryisms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, Burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chronic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, Dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic ateriosclerotic disease, Diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's Syndrome in middle age, drug- induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, epstein-barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hematophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallerrorden-Spatz disease, hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis (A), His bundle arrythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement disorders, hypersensitity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, Asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza a, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia- reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions ofthe corticospinal system, lipedema, liver

transplant rejection, lymphederma, malaria, malignamt Lymphoma, malignant histiocytosis, malignant melanoma, meningitis, meningococcemia, metabolic/idiopathic diseases, migraine headache, mitochondrial multi.system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel DejerineThomas Shi-Drager and Machado-Joseph), myasthenia gravis, mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodyplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic I muscular atrophies, neutropenic fever, non-hodgkins lymphoma, occlusion of the abdominal aorta and its branches, occlusive arterial disorders, okt3 therapy, orchitis/epidydimitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome l, hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherlosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiotomy syndrome, preeclampsia, Progressive supranucleo Palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynoud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia ofLewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions ofthe cerebellum, Subacute sclerosing panencephalitis, Syncope, syphilis of the cardiovascular system, systemic anaphalaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, traumalhemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, ,vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke- Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue, carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract (including cervix, uterus, and ovaries as

well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma), tumors ofthe brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas), solid tumors arising from hematopoietic malignancies such as leukemias, and lymphomas (both Hodgkin's and non-Hodgkin's lymphomas).

15. The use of claim 13 or 14, wherein the medicament is administered parenterally, subcutaneously, intramuscularly, intravenously, intrarticularly, intrabronchially, intraabdominally, intracapsularly, intracartilaginously, intracavitarily, intracelially, intracerebellarly, intracerebroventricularly, intracolically, intracervically, intragastrically, intrahepatically, intramyocardially, intraosteally, intrapelvically, intrapericardially, intraperitoneally, intrapleurally, intraprostatically, intrapulmonarily, intrarectally, intrarenally, intraretinally, intraspinally, intrasynovially, intrathoracically intrauterine, intravesically, bolusly, vaginally, rectally, buccally, sublingually, intranasally, and/or transdermally.

16. An isolated nucleic acid encoding a binding protein according to any one of claims 1-7.

17. A vector comprising an isolated nucleic acid according to claim 16, wherein the vector is optionally selected from the group consisting of pcDNA, pTT, pTT3, pEFBOS, pBV, pJV, pcDNA3.1 TOPO, pEF6 TOPO, pHybE and pBJ.

18. A host cell comprising the vector according to claim 17, wherein the host cell is optionally selected from the group consisting of a prokaryotic cell, an E.Coli, a eukaryotic cell, a protist cell, animal cell, plant cell, fungal cell, a mammalian cell, a yeast cell, an avian cell, an insect cell, a CHO cell, a COS cell, a S. cerevisiae cell, and an Sf9 cell.

19. A method of producing a binding protein, comprising culturing the host cell of claim 18 in culture medium under conditions sufficient to produce the binding protein.