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

In one embodiment the ~~invention~~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

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 ~~invention~~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 ~~invention~~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 ~~invention~~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

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 ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~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

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 ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~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,

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 ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~disclosure provides at least one anti-idiotype antibody to at least one binding protein of the present ~~invention~~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 ~~invention~~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.