



- (51) **International Patent Classification:**
C07K 16/28 (2006.01) *C07K 16/30* (2006.01)
- (21) **International Application Number:**
PCT/GB2012/052219
- (22) **International Filing Date:**
7 September 2012 (07.09.2012)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
1115529.8 8 September 2011 (08.09.2011) GB
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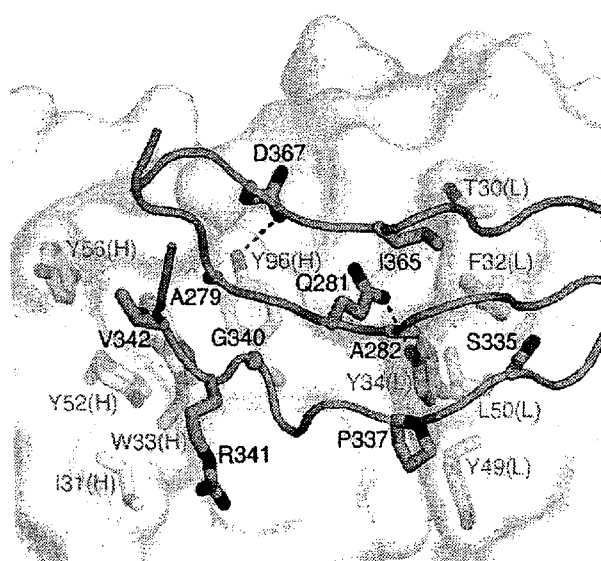
(81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

[Continued on next page]

- (54) **Title:** ANTI DDR1 ANTIBODIES, THEIR USES AND METHODS IDENTIFYING THEM

Figure 8



(57) **Abstract:** An antibody that specifically binds to the extracellular domain of human discoidin domain receptor 1 (DDR1) outside the discoidin domain, and which reduces or blocks collagen- induced autophosphorylation of DDR1 without preventing collagen binding to DDR1. Preferably, the antibody binds to the DS-like domain of human DDR1.

WO 2013/034933 A1



TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, **Published:**
ML, MR, NE, SN, TD, TG).

— *with international search report (Art. 21(3))*

ANTI DDR1 ANTIBODIES, THEIR USES AND METHODS IDENTIFYING THEM

The present invention generally relates to agents that target discoidin domain receptor 1, and more particularly to agents that bind to the DS-like domain of discoidin domain receptor 1. The invention also relates to methods, uses, kits, compounds and compositions comprising such agents.

Receptor tyrosine kinases are the targets of many anti-cancer therapies. Small-molecule drugs are used to target their intracellular portion, and more recently, monoclonal antibodies against their extracellular regions have been developed for clinical use. Since monoclonal antibodies selectively target tumour cells, their use minimises the cytotoxic side effects caused by traditional chemotherapeutics. Monoclonal antibodies are often non-mutagenic and are associated with few long-term adverse events, making them attractive therapeutic agents. Monoclonal antibodies against receptor tyrosine kinases have become critical components of clinical treatments for a variety of disorders, especially in oncology. Established monoclonal antibody-based therapies are routinely used for breast cancer treatment and haematological malignancies, and are in development for other cancers, such as colorectal, lung and ovarian cancers, for which new molecular targets are being sought.

Discoidin domain receptor 1 (DDR1) is a receptor tyrosine kinase that is a promising therapeutic target for a number of aggressive cancers. Numerous studies have linked DDR1 to tumour formation. In particular, fast growing, invasive tumours often show enhanced DDR1 expression and there is good evidence that cancer invasion and metastasis are mediated by DDR1. High level of DDR1 expression was correlated with advanced tumour stage and significantly poorer survival in several cohorts of patients with brain, breast, lung and ovarian cancer. In lung cancer, DDR1 was found to be among the top 10 activated kinases, both in patient samples and in cell lines. Furthermore, DDR1 is mutated in certain lung cancers and leukaemia. In addition to cancer cell invasion and metastasis, DDR1 contributes to cancer cell survival. Several studies have shown that depletion of DDR1 blocked cancer cell invasion, in both *in vitro* and *in vivo* models, indicating that DDR1 is a valid clinical target in cancer therapy.

DDR1 is a collagen receptor that plays a key role in cell attachment, migration, survival and proliferation. DDR1 mediates disease progression, particularly tumorigenesis, but also certain inflammatory and fibrotic disorders and atherosclerosis. Collagen binding to

DDR1 results in enhanced DDR1 kinase activity, which is manifested as increased autophosphorylation.

5 The extracellular domain of DDR1 consists of two protein modules, the discoidin domain and the discoidin-like (DS-like) domain. The discoidin domain fully contains the DDR1 ligand-binding site, which is located at the "top" of the domain.

10 US patent publication no. 2009/0142345 describes DDR1 neutralising antibodies, and their use in treating cancer. The entire disclosure of US 2009/0142345 relating to the role and expression of DDR1 in cancer, the production of anti-DDR1 antibodies, and the use of anti-DDR1 antibodies in the treatment of cancer, is incorporated herein in its entirety by reference.

15 US Patent No. 6,855,076 describes DDR1 activating antibodies for use in enhancing the maturation of immature macrophage or dendritic cells.

20 WO 2010/019702 describes the use of neutralising antibodies against DDR1, and more specifically antibodies that bind to the ligand binding site of DDR1 and which prevent or inhibit collagen binding to DDR1. The entire disclosure of WO 2010/019702 relating to the use of anti-DDR1 antibodies is incorporated herein in its entirety by reference.

25 We produced seven antagonistic monoclonal antibodies (mAbs) against human DDR1 that are specific for human DDR1 and block DDR1 activation that results from collagen binding. When these mAbs are added to cells expressing DDR1, collagen is no longer able to induce DDR1 autophosphorylation.

30 Interestingly and unexpectedly, all our anti-DDR1 mAbs bind the discoidin-like domain, and not the discoidin domain of DDR1. Epitope mapping showed that their epitopes are located in at least three regions of the discoidin-like domain located towards the "bottom" of this domain. In keeping with their binding epitopes being distinct from the ligand-binding site, we have shown that these anti-DDR1 mAbs do not block the binding of collagen to DDR1. We tested the Fab portion of a subset of the mAbs, and these fragments were also active as DDR1 blocking reagents. In summary, these anti-DDR1 mAbs block collagen-induced DDR1 phosphorylation but they do not inhibit collagen
35 binding to DDR1. Without wishing to be bound by any theory, we consider that the mechanism of action may involve the stabilisation of an inactive DDR1 conformation.

We have also shown these monoclonal antibodies to block collagen-induced DDR1 phosphorylation in several cancer cell lines. Thus, these antibodies have significant potential for clinical use.

5 Accordingly, a first aspect of the invention provides an antibody that specifically binds to the extracellular domain of human discoidin domain receptor 1 (DDR1) outside the discoidin domain, and which reduces or blocks collagen-induced autophosphorylation of DDR1 without preventing collagen binding to DDR1.

10 Antibodies that specifically or selectively bind to DDR1 are also referred to as anti-DDR1 antibodies. Such antibodies will bind their target with a greater affinity than for an irrelevant polypeptide, such as human serum albumin (HSA). Preferably, the antibody binds the DDR1 with at least 5, or at least 10 or at least 50 times greater affinity than for the irrelevant polypeptide. More preferably, the antibody molecule binds the DDR1 with
15 at least 100, or at least 1,000, or at least 10,000 times greater affinity than for the irrelevant polypeptide. Such binding may be determined by methods well known in the art, such as one of the Biacore® systems.

It is preferred if the antibodies have an affinity for DDR1 of at least 10^{-7} M and more
20 preferably 10^{-8} M, although antibodies with higher affinities, e.g. 10^{-9} M, or higher, may be even more preferred.

Typically, the antibody that selectively binds to the DDR1 polypeptide binds to the DS-like domain of DDR1, or to specific epitopes therein.

25

Preferably, when the antibody is administered to an individual, the antibody binds to DDR1 or to the specified portion thereof, with a greater affinity than for DDR2. Preferably, the antibody binds to (the specified portion of) DDR1 with at least 2, or at least 5, or at least 10 or at least 50 times greater affinity than for DDR2 in the individual.
30 More preferably, the agent binds the DDR1 (at the specified domain) with at least 100, or at least 1,000, or at least 10,000 times greater affinity than for DDR2 in the individual. Further preferably, the antibody molecule selectively binds the DDR1 without significantly binding DDR2, or only binds to DDR2 at undetectable levels.

35 In an embodiment, when the antibody is administered to an individual, the antibody binds to DDR1 or to the specified portion thereof, with a greater affinity than for any other molecule in the individual. Preferably, the antibody binds to the specified portion of

DDR1 with at least 2, or at least 5, or at least 10 or at least 50 times greater affinity than for any other molecule in the individual. More preferably, the agent binds the DDR1 at the specified domain with at least 100, or at least 1,000, or at least 10,000 times greater affinity than any other molecule in the individual. Preferably, the antibody molecule
5 selectively binds the DDR1 without significantly binding other polypeptides in the body, such as for example other RTKs, although in practice there may be some cross reactivity that does not affect the intended use of the antibody.

By "antibody" we include substantially intact antibody molecules, as well as antigen-
10 binding fragments of antibodies, chimaeric antibodies, humanised antibodies, human antibodies (wherein at least one amino acid is mutated relative to the naturally occurring human antibodies), single chain antibodies, bispecific antibodies, antibody heavy chains, antibody light chains, homodimers and heterodimers of antibody heavy and/or light chains, and antigen binding fragments and derivatives of the same. We also include
15 fusions or derivatives thereof. For example, the antibody or antigen-binding fragment, or fusion or derivative thereof, may comprise, consist or consist essentially of an intact antibody. By "consist essentially of" we mean that the antibody or antigen-binding fragment, fusion or derivative thereof consists of a portion of an intact antibody sufficient to retain binding specificity for the specified region or epitope of DDR1.

20

The term 'antibody' also includes all classes of antibodies, including IgG, IgA, IgM, IgD and IgE. Thus, the antibody may be an IgG molecule, such as an IgG1, IgG2, IgG3, or IgG4 molecule.

25 Preferably, the antibody is an IgG antibody, for example, an IgG2 or IgG4 antibody. In one preferred embodiment, the antibody is an IgG4 antibody in which the Serine amino acid at position 241 has been substituted with a Proline residue (*i.e.* S241P) – such a substitution is known to stabilise the disulphide bridges in IgG4 molecule, resulting in a more stable antibody (Angal *et al.*, 1993, *Mol. Immunol.*, 30:105-8).

30

It will be appreciated by persons skilled in the art that the binding specificity of an antibody or antigen binding fragment thereof is conferred by the presence of Complementarity Determining Regions (CDRs) within the variable regions of the constituent heavy and light chains.

35

The variable heavy (V_H) and variable light (V_L) domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further

confirmation was found by "humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent-parented antibody (Morrison *et al* (1984) *Proc. Natl. Acad. Sci. USA* 81, 6851-6855).

5

Antigenic specificity is conferred by variable domains and is independent of the constant domains, as known from experiments involving the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better *et al* (1988) *Science* 240, 1041); Fv molecules (Skerra *et al* (1988) *Science* 240, 1038); single-chain Fv (ScFv) molecules where the V_H and V_L partner domains are linked via a flexible oligopeptide (Bird *et al* (1988) *Science* 242, 423; Huston *et al* (1988) *Proc. Natl. Acad. Sci. USA* 85, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward *et al* (1989) *Nature* 341, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) *Nature* 349, 293-299.

15

Thus, by "antigen-binding fragment" we mean a functional fragment of an antibody that is capable of binding to the specified region or epitope of DDR1.

Exemplary antigen-binding fragments of the invention may be selected from the group consisting of Fv fragments (*e.g.* single chain Fv and disulphide-bonded Fv), and Fab-like fragments (*e.g.* Fab fragments, Fab' fragments and F(ab)₂ fragments).

20

In a preferred embodiment, the antigen-binding fragment is an scFv.

25

The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments may lead to improved pharmacological properties, such as better penetration of solid tissue. Moreover, antigen-binding fragments such as Fab, Fv, ScFv and dAb antibody fragments can be expressed in and secreted from *E. coli* or yeast, thus allowing the facile production of large amounts of the said fragments.

30

Also included within the scope of the invention are modified versions of antibodies and antigen-binding fragments thereof, *e.g.* modified by the covalent attachment of polyethylene glycol or other suitable polymer.

35

Methods of generating antibodies and antibody fragments are well known in the art. For example, antibodies may be generated via any one of several methods which employ

induction of *in vivo* production of antibody molecules, screening of immunoglobulin libraries (Orlandi. *et al*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86:3833-3837; Winter *et al.*, 1991, *Nature* 349:293-299) or generation of monoclonal antibody molecules by cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the Epstein-Barr virus (EBV)-hybridoma technique (Kohler
5 *et al.*, 1975. *Nature* 256:495-497; Kozbor *et al.*, 1985. *J. Immunol. Methods* 81:31-42; Cote *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80:2026-2030; Cole *et al.*, 1984. *Mol. Cell. Biol.* 62:109-120).

10 The antibody or antigen-binding fragment or derivative thereof may be produced by recombinant means.

Preferably, the antibody is a monoclonal antibody. Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in
15 "*Monoclonal Antibodies: A manual of techniques*", H Zola (CRC Press, 1988) and in "*Monoclonal Hybridoma Antibodies: Techniques and Applications*", J G R Hurrell (CRC Press, 1982) , which are incorporated herein by reference.

Antibody fragments can also be obtained using methods well known in the art (see, for
20 example, Harlow & Lane, 1988, "*Antibodies: A Laboratory Manual*", Cold Spring Harbor Laboratory, New York, which is incorporated herein by reference). For example, antibody fragments according to the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* or mammalian cells (e.g. Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the
25 fragment. Alternatively, antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. Alternatively, antibody fragments can be obtained by cell-free *in vitro* expression, as is known in the art.

In other embodiments, the antibody may be a single-domain antibody, such as a
30 Nanobody. Such antibodies are known to exist in camelids (*Curr. Opin. Pharmacol.*, 8, (2008), 600-608) and sharks (e.g. IgNAR; *Curr. Opin. Pharmacol.*, 8, (2008), 600-608). Nanobodies[®] are antibody-derived therapeutic proteins that contain the structural and functional properties of naturally-occurring heavy-chain antibodies. The Nanobody[®] technology was developed following the discovery that camelidae (camels and llamas)
35 possess fully functional antibodies that lack light chains. These heavy-chain antibodies contain a single variable domain (VHH) and two constant domains (C_H2 and C_H3). The cloned and isolated VHH domain is a perfectly stable polypeptide harbouring the full

antigen-binding capacity of the original heavy-chain antibody. These VHH domains with their unique structural and functional properties form the basis of Nanobodies[®]. They combine the advantages of conventional antibodies (high target specificity, high target affinity and low inherent toxicity) with important features of small molecule drugs (the ability to inhibit enzymes and access receptor clefts). Furthermore, they are stable, have the potential to be administered by means other than injection, are easier to manufacture, and can be humanised. (See, for example US 5,840,526; US 5,874,541; US 6,005,079, US 6,765,087; EP 1 589 107; WO 97/34103; WO97/49805; US 5,800,988; US 5,874, 541 and US 6,015,695).

Other preferred antibody fragments include isolated heavy-chain variable (V_H) regions or isolated light-chain (V_L) regions, for example from human antibodies (*Curr. Opin. Pharmacol.*, 8, (2008), 600-608), and iMabs (WO 03/050283).

By 'fusion' we include an antibody or antigen-binding fragment (as defined herein) fused to any other polypeptide. For example, the antibody or antigen-binding fragment may be fused to a polypeptide such as glutathione-S-transferase (GST) or protein A in order to facilitate its purification. Examples of such fusions are well known to those skilled in the art. Similarly, the said antibody or antigen-binding fragment may be fused to an oligo-histidine tag such as His6 or to an epitope recognised by a further antibody (such as the well-known Myc tag epitope).

The fusion may comprise a further portion which confers a desirable feature on the antibody or antigen-binding fragment of the invention; for example, the portion may be useful in detecting or isolating the antibody or antigen-binding fragment, or promoting cellular uptake of the antibody or antigen-binding fragment. The portion may be, for example, a biotin moiety, a radioactive moiety, a fluorescent moiety, for example a small fluorophore or a green fluorescent protein (GFP) fluorophore, as well known to those skilled in the art. The moiety may be an immunogenic tag, for example a Myc tag, as known to those skilled in the art, or may be a lipophilic molecule or polypeptide domain that is capable of promoting cellular uptake, as known to those skilled in the art.

Methods for conjugating additional moieties to an antibody (or a fusion or derivative thereof) are well known in the art. Exemplary methods are described in Bioconjugate Techniques, 2nd Edition (2008); Hermanson (Academic Press, Inc.) and in Veronese *et al.*, (1999; *Farmaco* 54(8): 497-516); Stayton *et al.*, (2005; *Orthod Craniofac Res* 8(3): 219-225); Schrama *et al.*, (2006; *Nat Rev Drug Discov* 5(2): 147-159); Doronina *et al.*

(2003; Nat Biotechnol 21(7): 778-784); Carter *et al.*, (2008; Cancer J 14(3): 154-169); Torchilin (2006; Annu Rev Biomed Eng 8: 343-375); Rihova (1998; Adv Drug Deliv Rev 29(3): 273-289); Goyal *et al.* (2005; Acta Pharm 55(1): 1-25); Chari (1998; Adv Drug Deliv Rev 31(1-2): 89-104); Garnett (2001; Adv Drug Deliv Rev 53(2): 171-216); Allen
5 (2002; Nat Rev Cancer 2(10): 750-763).

The antibody or antigen-binding fragment of the invention may comprise one or more amino acids which have been modified or derivatised.

10 Chemical derivatives of one or more amino acids may be achieved by reaction with a functional side group. Such derivatised molecules include, for example, those molecules in which free amino groups have been derivatised to form amine hydrochlorides, *p*-toluene sulphonyl groups, carboxybenzoxy groups, *t*-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatised to form
15 salts, methyl and ethyl esters or other types of esters and hydrazides. Free hydroxyl groups may be derivatised to form O-acyl or O-alkyl derivatives. Also included as chemical derivatives are those peptides which contain naturally occurring amino acid derivatives of the twenty standard amino acids. For example: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine
20 may be substituted for histidine; homoserine may be substituted for serine and ornithine for lysine. Derivatives also include peptides containing one or more additions or deletions as long as the requisite activity is maintained. Other included modifications are amidation, amino terminal acylation (e.g. acetylation or thioglycolic acid amidation), terminal carboxylamidation (e.g. with ammonia or methylamine), and the like terminal
25 modifications.

It will be further appreciated by persons skilled in the art that peptidomimetic compounds may also be useful. Thus, the present invention includes peptidomimetic compounds which are capable of binding to the specified region or epitope of DDR1. The term
30 'peptidomimetic' refers to a compound that mimics the conformation and desirable features of a particular peptide as a therapeutic agent.

For example, the antibody, antigen-binding fragment, fusion or derivative thereof of the invention include not only molecules in which amino acid residues are joined by peptide
35 (-CO-NH-) linkages but also molecules in which the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Meziere *et al.* (1997) *J. Immunol.* 159, 3230-3237, which is

incorporated herein by reference. This approach involves making pseudo-peptides containing changes involving the backbone, and not the orientation of side chains. Retro-inverse peptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis. Alternatively, the antibody, antigen-binding
5 fragment, fusion or derivative thereof of the invention may be a peptidomimetic compound wherein one or more of the amino acid residues are linked by a $-\gamma(\text{CH}_2\text{NH})-$ bond in place of the conventional amide linkage.

In a further alternative, the peptide bond may be dispensed with altogether provided that
10 an appropriate linker moiety which retains the spacing between the carbon atoms of the amino acid residues is used; it may be advantageous for the linker moiety to have substantially the same charge distribution and substantially the same planarity as a peptide bond.

It will be appreciated that the antibody, antigen-binding fragment, variant, fusion or
15 derivative thereof of the invention may conveniently be blocked at its N- or C-terminus so as to help reduce susceptibility to exo-proteolytic digestion.

A variety of un-coded or modified amino acids such as D-amino acids and N-methyl
20 amino acids have also been used to modify mammalian peptides. In addition, a presumed bioactive conformation may be stabilised by a covalent modification, such as cyclisation or by incorporation of lactam or other types of bridges, for example see Veber *et al.*, 1978, *Proc. Natl. Acad. Sci. USA* 75:2636 and Thursell *et al.*, 1983, *Biochem. Biophys. Res. Comm.* 111:166, which are incorporated herein by reference.

25

A common theme among many of the synthetic strategies has been the introduction of some cyclic moiety into a peptide-based framework. The cyclic moiety restricts the conformational space of the peptide structure and this frequently results in an increased specificity of the peptide for a particular biological receptor. An added advantage of this
30 strategy is that the introduction of a cyclic moiety into a peptide may also result in the peptide having a diminished sensitivity to cellular peptidases. Thus, exemplary antibody, antigen-binding fragment, fusion or derivative thereof of the invention may comprise terminal modifications as is known in the art, to reduce susceptibility by proteinase digestion and therefore to prolong their half-life in biological fluids where proteases may
35 be present.

Advantageously, the invention provides an agent wherein the antibody or antigen-binding fragment thereof is human or humanised.

It will be appreciated by persons skilled in the art that, for human therapy or diagnostics, humanised antibodies may be used. Humanised forms of non-human (e.g. murine) antibodies are genetically engineered chimaeric antibodies or antibody fragments having minimal-portions derived from non-human antibodies. Humanised antibodies include antibodies in which complementary determining regions of a human antibody (recipient antibody) are replaced by residues from a complementary determining region of a non human species (donor antibody) such as mouse, rat or rabbit having the desired functionality. In some instances, Fv framework residues of the human antibody are replaced by corresponding non-human residues. Humanised antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported Complementarity Determining Region (CDR) or framework sequences. In general, the humanised antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the complementarity determining regions correspond to those of a non-human antibody and all, or substantially all, of the framework regions correspond to those of a relevant human consensus sequence. Humanised antibodies optimally also include at least a portion of an antibody constant region, such as an Fc region, typically derived from a human antibody (see, for example, Jones *et al.*, 1986, *Nature* 321:522-525; Riechmann *et al.*, 1988, *Nature* 332:323-329; Presta, 1992, *Curr. Op. Struct. Biol.* 2:593-596, which are incorporated herein by reference).

Methods for humanising non-human antibodies are well known in the art. Generally, the humanised antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues, often referred to as imported residues, are typically taken from an imported variable domain. Humanisation can be essentially performed as described (see, for example, Jones *et al.*, 1986, *Nature* 321:522-525; Reichmann *et al.*, 1988, *Nature* 332:323-327; Verhoeyen *et al.*, 1988, *Science* 239:1534-1536; US 4,816,567, which are incorporated herein by reference) by substituting human complementarity determining regions with corresponding rodent complementarity determining regions. Accordingly, such humanised antibodies are chimaeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanised antibodies may be typically human antibodies in which some

complementarity determining region residues and possibly some framework residues are substituted by residues from analogous sites in rodent antibodies.

Completely human antibodies may be produced using recombinant technologies.

5 Typically large libraries comprising billions of different antibodies are used. In contrast to the previous technologies employing chimaerisation or humanisation of e.g. murine antibodies this technology does not rely on immunisation of animals to generate the specific antibody. Instead the recombinant libraries comprise a huge number of pre-made antibody variants wherein it is likely that the library will have at least one antibody

10 specific for any antigen. Thus, using such libraries, an existing antibody having the desired binding characteristics can be identified. In order to find the good binder in a library in an efficient manner, various systems where phenotype i.e. the antibody or antibody fragment is linked to its genotype i.e. the encoding gene have been devised. The most commonly used such system is the so called phage display system where

15 antibody fragments are expressed, displayed, as fusions with phage coat proteins on the surface of filamentous phage particles, while simultaneously carrying the genetic information encoding the displayed molecule (McCafferty *et al*, 1990, *Nature* **348**: 552-554). Phage displaying antibody fragments specific for a particular antigen may be selected through binding to the antigen in question. Isolated phage may then be

20 amplified and the gene encoding the selected antibody variable domains may optionally be transferred to other antibody formats, such as e.g. full-length immunoglobulin, and expressed in high amounts using appropriate vectors and host cells well known in the art. Alternatively, the "human" antibodies can be made by immunising transgenic mice which contain, in essence, human immunoglobulin genes (Vaughan *et al* (1998) *Nature*

25 *Biotechnol.* **16**, 535-539).

The format of displayed antibody specificities on phage particles may differ. The most commonly used formats are Fab (Griffiths *et al*, 1994, *EMBO J.* **13**: 3245-3260) and single chain (scFv) (Hoogenboom *et al*, 1992, *J Mol Biol.* **227**: 381-388) both comprising

30 the variable antigen binding domains of antibodies. The single chain format is composed of a variable heavy domain (V_H) linked to a variable light domain (V_L) *via* a flexible linker (US 4,946,778). Before use as a therapeutic agent, the antibody may be transferred to a soluble format e.g. Fab or scFv and analysed as such. In later steps the antibody fragment identified to have desirable characteristics may be transferred into yet other

35 formats such as full-length antibodies.

WO 98/32845 and Soderlind *et al* (2000) *Nature BioTechnol.* **18**: 852-856 describe technology for the generation of variability in antibody libraries. Antibody fragments derived from this library all have the same framework regions and only differ in their CDRs. Since the framework regions are of germline sequence the immunogenicity of antibodies derived from the library, or similar libraries produced using the same technology, are expected to be particularly low (Soderlind *et al*, 2000). This property is of great value for therapeutic antibodies, reducing the risk that the patient forms antibodies to the administered antibody, thereby reducing risks for allergic reactions, the occurrence of blocking antibodies, and allowing a long plasma half-life of the antibody.

Thus, when developing therapeutic antibodies to be used in humans, modern recombinant library technology (Soderlind *et al*, 2001, *Comb. Chem. & High Throughput Screen.* **4**: 409-416) is often used in preference to the earlier hybridoma technology.

Once suitable antibodies are obtained, they may be tested for activity, such as binding specificity or a biological activity of the antibody, for example by ELISA, immunohistochemistry, flow cytometry, immunoprecipitation, Western blots, *etc.* The biological activity may be tested in different assays with readouts for that particular feature.

In a preferred embodiment, the antibody, antigen-binding fragment, fusion or derivative thereof is in an isolated and/or purified form.

In one embodiment, the antibody is a non-naturally occurring antibody. Of course, where the antibody is a naturally occurring antibody, it is provided in an isolated form (*i.e.* distinct from that in which it is found in nature).

The sequence of human DDR1, Isoform 1, taken from GenBank Accession No. Q08345, is as follows:

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      10      20      30      40      50      60
5  MGPEALSSLL LLLLVASGDA DMKGHFDPAK CRYALGMQDR TIPDSDISAS SSWSDSTAAR

      70      80      90     100     110     120
   HSRLESSDGD GAWCPAGSVF PKEEEYLQVD LQRLHLVALV GTQGRHAGGL GKEFSRSYRL

      130     140     150     160     170     180
10  RYSRDGRRWM GWKDRWGQEV ISGNEDPEGV VLKDLGPPMV ARLVRFYPRA DRVMSVCLRW

      190     200     210     220     230     240
   ELYGCLWRDG LLSYTAPVGQ TMYLSEAVYL NDSTYDGHTV GGLQYGGLGQ LADGVVGLDD

      250     260     270     280     290     300
15  FRKSQELRVW PGYDYVGWSN HSFSSGYVEM EFEFDRLRAF QAMQVHCNNM HTLGARLPGG

      310     320     330     340     350     360
   VECRFRRGPA MAWEGEPMRH NLGGNLGDPR ARAVSVP LGG RVARFLQCRF LFAGPWLLFS

20  EISFISDVVN NSSPALGGTF PPAPWWPPGP PPTNFSSLEL EPRGQQPVAK AEGSPTAILI

      370     380     390     400     410     420
   GCLVAIILL LLIIALMLWR LHWRRLLSKA ERRVLEELT VHLSVPGDTI LINNRPGPRE

      430     440     450     460     470     480
25  PPPYQEPRPR GNPPHSAPCV PNGSALLLSN PAYRLLLATY ARPPRGPGPP TPAWAKPTNT

      490     500     510     520     530     540
   QAYSGDYMEP EKPGAPLLPP PPQNSVPHYA EADIVTLQGV TGGNTYAVPA LPPGAVGDGP

      550     560     570     580     590     600
30  PRVDFPRSR LRFKEKLGEQ FGEVHLCEVD SPQDLVSLDF PLNVRKGHPL LVAVKILRPD

      610     620     630     640     650     660
35  ATKNNRNDFL KEVKIMSRLK DPNIIRLLGV CVQDDPLCMI TDYMENGDLN QFLSAHQLED

      670     680     690     700     710     720
   KAAEGAPGDG QAAQGPTISY PMLLHVAAQI ASGMRYLATL NFVHRDLATR NCLVGENFTI

      730     740     750     760     770     780
40  KIADFGMSRN LYAGDYRVQ GRAVLPIRWM AWECILMGKF TTASDVWAFG VTLWEVLMMLC

      790     800     810     820     830     840
   RAQPFQQLTD EQVIENAGEF FRDQGRQVYL SRPPACPQGL YELMLRCWSR ESEQRPFFSQ

      850     860     870     880     890     900
45  LHRFLAEDAL NTV

      910
50

```

Typically and preferably, by "discoidin domain receptor 1" or "DDR1", we refer to the human DDR1, Isoform 1, whose sequence taken from Q08345 is listed immediately above.

However, by "discoidin domain receptor 1" or "DDR1", we also include isoforms and variants of the DDR1 protein, including DDR1a, DDR1b, DDR1c, DDR1d and DDR1e, which all contain the DS-like domain, unless the context demands otherwise.

- 5 DDR1, and its phosphorylation and activation by collagen types I, II, III, IV and V, is described in US patent publication no. 2003/0070184, as are methods for determining whether or not collagen binds to the DDR1 in the presence of the anti-DDR1 antibody of the invention. The entire disclosure of US 2003/0070184 relating to the binding of collagens to DDR, and to the activation of DDR1, is incorporated herein by reference.

10

Residues 1 to 18 of human DDR1 are the signal peptide; the extracellular domain is from residues 19 to 416, the transmembrane domain is from residue 417 to about residue 439 (Noordeen *et al*, J. Biol. Chem, **281**:22744-22751), and the cytoplasmic domain is from about residues 440 to residue 913. Within the extracellular region, the discoidin domain is from residues 31-185, and the discoidin-like domain (DS-like) is from residues 186 to 367.

15

Thus by the DS-like domain of DDR1 we mean the sequence from residues 187 to 367 of DDR1 isoform 1 whose sequence, taken from Q08345, is listed above.

20

In a preferred embodiment, it is preferred that the antibody which specifically binds to the extracellular domain of human DDR1 outside the discoidin domain, and which reduces or blocks collagen-induced autophosphorylation of DDR1 without preventing collagen binding to DDR1, specifically binds to the DS-like domain of human DDR1.

25

As discussed herein, important residues involved in the binding of 3E3 to the DS-like domain of human DDR1 are residues Ala279, Gln281, Ala282, Ser335, Pro337, Gly340, Arg341, Val342, Ile365 and Asp367.

- 30 Thus, in an embodiment, it may be preferred that the antibody specifically binds to the DDR1 epitope defined by the amino acid residues Ala279, Gln281, Ala282, Ser335, Pro337, Gly340, Arg341, Val342, Ile365 and Asp367.

By an antibody that specifically binds to this DDR1 epitope, we mean that the antibody binds to DDR1 through at least one of these amino acid residues. Preferably, the antibody binds to the DDR1 through at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 of Ala279, Gln281, Ala282, Ser335, Pro337, Gly340,

35

Arg341, Val342, Ile365 and Asp367. In a specific embodiment, the antibody binds to DDR1 through all ten of these residues.

5 By "epitope" we mean a site of a molecule to which an antibody binds, *i.e.* a molecular region of an antigen. An epitope may be a linear epitope, which is determined by *e.g.* the amino acid sequence, *i.e.* the primary structure, or a three-dimensional epitope, defined by the secondary structure, *e.g.* folding of a peptide chain into beta sheet or alpha helical, or by the tertiary structure, *e.g.* way which helices or sheets are folded or arranged to give a three-dimensional structure, of an antigen.

10

In alternative embodiments, it may be preferred that the antibody specifically binds to the DDR1 epitope encompassed by the amino acid residues Tyr203 to Tyr209 (as defined in the DDR1-epi1 mutation in Example 1), or to the DDR1 epitope defined by the amino acid residues Met318, Asn321 and Asn325 (as defined in the DDR1-epi6 mutation in
15 Example 1).

By an antibody that specifically binds to the DDR1 epitope encompassed by amino acid residues Tyr203 to Tyr209, we mean that the antibody binds to DDR1 through at least one of the seven amino acid residues encompassed by Tyr203 to Tyr209. Preferably,
20 the antibody binds to the DDR1 through at least 2, at least 3, at least 4, at least 5, or at least 6 of the seven amino acid residues encompassed by Tyr203 to Tyr209. In a specific embodiment, the antibody binds to DDR1 through all seven of the residues encompassed by Tyr203 to Tyr209.

25 By an antibody that specifically binds to the DDR1 epitope defined by amino acid residues Met318, Asn321 and Asn325, we mean that the antibody binds to DDR1 through at least one of these three amino acid residues. Preferably, the antibody binds to the DDR1 through two of these residues, for example Met318 and Asn321, Met318 and Asn325, or Asn321 and Asn325. In a specific preferred embodiment, the antibody
30 binds to DDR1 through all three of Met318, Asn321 and Asn325.

In certain preferred embodiments, it is preferred that the antibody is capable of inhibiting growth of a tumour cell. This inhibition may be *in vivo* or *in vitro*, and can be determined using methods very well known to the skilled person. The tumour cell may be, for
35 example, a breast, colorectal, hepatic, renal, lung, pancreatic, ovarian, prostate or head and neck tumour cell.

In some embodiments, it is preferred that the antibody is capable of reducing the frequency of cancer stem cells in a tumour. This inhibition can be determined using methods described in WO 2010/019702 and known to the skilled person.

- 5 In further embodiments, the invention provides an antibody that specifically binds to the discoidin-like domain of human DDR1, wherein the antibody comprises:
- a heavy chain CDR1 comprising the amino acid sequence SGYTFSISW,
 - a heavy chain CDR2 comprising the amino acid sequence YPSGGY, and/or
 - a heavy chain CDR3 comprising the amino acid sequence RGYGHLEY,
- 10 or a variant of any of these sequences comprising 1, 2 or 3 amino acid substitutions.

This antibody may further comprise:

- a light chain CDR1 comprising the amino acid sequence SSSVTF,
 - a light chain CDR2 comprising the amino acid sequence YLTSN, and/or
 - 15 a light chain CDR3 comprising the amino acid sequence QWSSNPYT,
- or a variant of any of these sequences comprising 1, 2 or 3 amino acid substitutions.

In yet another embodiment, the invention provides an antibody that specifically binds to the discoidin-like domain of human DDR1, wherein the antibody comprises:

- 20 a light chain CDR1 comprising the amino acid sequence SSSVTF,
- a light chain CDR2 comprising the amino acid sequence YLTSN, and/or
 - a light chain CDR3 comprising the amino acid sequence QWSSNPYT,
- or a variant of any of these sequences comprising 1, 2 or 3 amino acid substitutions..

- 25 In still another embodiment, the invention provides an antibody that specifically binds to the discoidin-like domain of human DDR1, wherein the antibody comprises:

- (a) a heavy chain CDR1 comprising the amino acid sequence SGYTFSISW,
 - a heavy chain CDR2 comprising the amino acid sequence YPSGGY, and/or
 - a heavy chain CDR3 comprising the amino acid sequence RGYGHLEY; and
 - 30 (b) a light chain CDR1 comprising the amino acid sequence SSSVTF,
 - a light chain CDR2 comprising the amino acid sequence YLTSN, and
 - a light chain CDR3 comprising the amino acid sequence QWSSNPYT,
- or a variant of any of these sequences comprising 1, 2 or 3 amino acid substitutions.

- 35 In a more specific embodiment, the invention provides an antibody that specifically binds to the discoidin-like domain of human DDR1, wherein the antibody comprises:

- (a) a heavy chain CDR1 comprising the amino acid sequence SGYTFSISW,

- a heavy chain CDR2 comprising the amino acid sequence YPSGGY, and
 a heavy chain CDR3 comprising the amino acid sequence RGYGHLEY; and
 (b) a light chain CDR1 comprising the amino acid sequence SSSVTF,
 a light chain CDR2 comprising the amino acid sequence YLTSN, and
 5 a light chain CDR3 comprising the amino acid sequence QWSSNPYT,
 or a variant of any of these sequences comprising 1 or 2 amino acid substitutions.

In a yet further embodiment, the invention provides an antibody that specifically binds to the discoidin-like domain of human DDR1, wherein the antibody comprises:

- 10 (a) a heavy chain variable region sequence
 QVQLQESGAELVRPGASVKLSCKASGYTFSISWINVVKQRPGQGLEWIGNIYPSGGYTN
 NQKFQDKATLTVDKSSNTAYIQLSSPTSEDSAVYYCTRGYGHLDYWGQGTLLTVSA; and/or
 (b) a light chain variable sequence,
 DIQLTQSPALMSASPGEKVTMTCSASSSVTFMYWYQKPRSSPKPWIYLTSLASGVPAR
 15 FSGSGSGTSYSLTISSEAEADAATYYCQWSSNPYTFGGGTKLEIK,
 or a variant of any of these sequences comprising 1, 2, 3, 4 or 5 amino acid
 substitutions.

- Preferably, the amino acid substitutions are conservative amino acid substitutions.
 20 Conservative substitutions providing functionally similar amino acids are well known in
 the art, and described for example in Table 1 of WO 2010/019702, which is incorporated
 herein by reference. Guidance concerning which amino acid changes are likely to be
 phenotypically silent can also be found in Bowie *et al.*, 1990, Science 247:1306-1310.
 Such conservatively modified variants are in addition to and do not exclude polymorphic
 25 variants, interspecies homologues, and alleles. Typical conservative substitution groups
 include: (1) Alanine (A), Glycine (G); (2) Aspartic acid (D), Glutamic acid (E); (3)
 Asparagine (N), Glutamine (Q); (4) Arginine (R), Lysine (K); (5) Isoleucine (I), Leucine
 (L), Methionine (M), Valine (V); (6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); (7)
 Serine (S), Threonine (T); and (8) Cysteine (C), Methionine (M) (see, e.g., Creighton,
 30 Proteins (1984)). As indicated, changes are typically of a minor nature that do not
 significantly affect the folding, binding or activity of the protein.

In another embodiment, the antibody comprises:

(a) a heavy chain sequence

QVQLQESGAELVRPGASVKLSCKASGYTFSISWINWVKQRPQGQLEWIGNIYPSGGYTN
 NQKFQDKATLTVDKSSNTAYIQLSSPTSSEDSAVYYCTRGYGHLDYWGQGTLVTVSAAKTT
 5 PPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTL
 SSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVP; and

(b) a kappa light chain sequence

DIQLTQSPALMSASPGEKVTMTCSASSSVTFMYWYQKPRSSPKPWIYLTSLASGVPAR
 FSGSGSGTSYSLTISSEMEADAATYYCQQWSSNPYTFGGGTKLEIKRADAAPTVSIFPPS
 10 SEQLTSGGASVVCFLNFPYKDIINVKWKIDGSRQNGVLNSWTDQDSKDYSTYSMSSTLTTL
 TKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC.

These are the sequences of 3E3 antibody listed in Figure 11.

- 15 In an embodiment, the invention also provides an antibody that is capable of competing with antibody 3E3, i.e., having the light and heavy chain sequences defined in Figure 11, for binding to the DS-like domain of human DDR1.

By "capable of competing" for binding to the DS-like domain of DDR1 we mean that the
 20 antibody is capable of inhibiting or otherwise interfering, at least in part, with the binding of an antibody molecule having these variable region sequences to the DS-like domain of DDR1. For example, the antibody may be capable of inhibiting the binding of an antibody molecule having these variable region sequences by at least 10%, for example at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 35% or even by 100%.
 25 Competitive binding may be determined by methods well known to those skilled in the art, such as ELISA and/or SPR as is very well known in the art. In an embodiment, the antibody is capable of binding to the same epitope as an antibody having the sequence shown in Figure 11.

- 30 Another aspect of the invention provides a binding moiety that specifically binds to:
 the human DDR1 epitope defined by the amino acid residues Ala279, Gln281, Ala282, Ser335, Pro337, Gly340, Arg341, Val342, Ile365 and Asp367, or
 the human DDR1 epitope encompassed by the amino acid residues Tyr203 to Tyr209, or
 35 the human DDR1 epitope defined by the amino acid residues Met318, Asn321 and Asn325.

Each of these three epitopes are within the discoidin-like domain of human DDR1.

By a binding moiety that specifically binds to a specified DDR1 epitope we mean an agent, e.g, a molecule or compound, capable of reversibly and/or irreversibly associating with and binding to at least one of the specified amino acid residues by covalent and/or apolar and/or ionic interaction.

Preferably, the binding moiety that specifically binds to the human DDR1 epitope defined by the amino acid residues Ala279, Gln281, Ala282, Ser335, Pro337, Gly340, Arg341, Val342, Ile365 and Asp367 binds to or associates with at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 of Ala279, Gln281, Ala282, Ser335, Pro337, Gly340, Arg341, Val342, Ile365 and Asp367. In a specific embodiment, the binding moiety binds to or associates with all ten of these residues.

Preferably, the binding moiety that specifically binds to the human DDR1 epitope encompassed by the amino acid residues Tyr203 to Tyr209 binds to or associates with at least 2, at least 3, at least 4, at least 5, or at least 6 of the seven amino acid residues encompassed by Tyr203 to Tyr209. In a specific embodiment, the binding moiety binds to or associates with DDR1 through all seven of the residues encompassed by Tyr203 to Tyr209.

Preferably, the binding moiety that specifically binds to the human DDR1 epitope defined by the amino acid residues Met318, Asn321 and Asn325 binds to or associates with at least two of these residues, for example Met318 and Asn321, Met318 and Asn325, or Asn321 and Asn325. In a specific embodiment, the binding moiety binds to or associates with DDR1 through all three of Met318, Asn321 and Asn325.

Preferably the binding moiety reduces or blocks collagen-induced autophosphorylation of DDR1 without preventing collagen binding to DDR1. Suitable methods for determining this are described herein and are known in the art.

In various embodiments, the binding moiety may be an antibody, an antibody mimic (for example, based upon a non-antibody scaffold), an RNA aptamer, a small molecule or a CovX-body.

Preferences for when the binding moiety is an antibody are described above.

In an alternative embodiment the binding moiety is an antibody mimic (such as a non-antibody scaffold). It will be appreciated that antibody mimics (for example, non-antibody scaffold structures that have a high degree of stability yet allow variability to be introduced at certain positions) may be used to create molecular libraries from which binding moieties can be derived. Those skilled in the arts of biochemistry will be familiar with many such molecules. Such molecules may be used as a binding moiety in the agent of the present invention.

Exemplary antibody mimics are discussed in Skerra *et al.* (2007, *Curr. Opin. Biotech.*, 18: 295-304) and include: affibodies (also called Trinectins; Nygren, 2008, *FEBS J*, 275, 2668-2676); CTLDs (also called Tetranectins; *Innovations Pharmac. Technol.* (2006), 27-30); adnectins (also called monobodies; *Meth. Mol. Biol.*, 352 (2007), 95-109); anticalins (*Drug Discovery Today* (2005), 10, 23-33); DARPin (ankyrins; *Nat. Biotechnol.* (2004), 22, 575-582); avimers (*Nat. Biotechnol.* (2005), 23, 1556-1561); microbodies (*FEBS J*, (2007), 274, 86-95); peptide aptamers (*Expert. Opin. Biol. Ther.* (2005), 5, 783-797); Kunitz domains (*J. Pharmacol. Exp. Ther.* (2006) 318, 803-809); affilins (*Trends. Biotechnol.* (2005), 23, 514-522).

Accordingly, it is preferred that the antibody mimic is selected from the group comprising or consisting of affibodies, tetranectins (CTLDs), adnectins (monobodies), anticalins, DARPin (ankyrins), avimers, iMabs, microbodies, peptide aptamers, Kunitz domains and affilins.

RNA aptamers represent a unique emerging class of therapeutic agents (Que-Gewirth *et al.*, *Gene Ther.* 74:283 (2007); Ireson *et al.*, *Mol. Cancer Ther.* 5:2957 (2006)). They are relatively short (12-30 nucleotide) single-stranded RNA oligonucleotides that assume a stable three-dimensional shape to tightly and specifically bind selected protein targets to elicit a biological response. In contrast to antisense oligonucleotides, RNA aptamers can effectively target extracellular targets. Like antibodies, aptamers possess binding affinities in the low nanomolar to picomolar range. In addition, aptamers are heat stable, non-immunogenic, and possess minimal inter-batch variability. Chemical modifications, such as amino or fluoro substitutions at the 2' position of pyrimidines, may reduce degradation by nucleases. The biodistribution and clearance of aptamers can also be altered by chemical addition of moieties such as polyethylene glycol and cholesterol.

Aptamers may be developed by iterative selection methods such as SELEX (systematic evolution of ligands by exponential enrichment) to specifically recognize and tightly bind their targets by means of well-defined complementary three-dimensional structures. Further, SELEX (and other such methods) allows selection from libraries to generate
5 high-affinity oligonucleotide ligands to purified biochemical targets. Recently, the aptamer pegaptanib was approved for the treatment of age-related macular degeneration (Wong et al, Lancet 370:204 (2007)). With regard to the field of oncology, the DNA aptamer GBI-10, derived from a human glioblastoma cell line, was recently demonstrated to bind tenascin-C (Daniels et al, Proc. Natl. Acad. Sci. USA 100:15416
10 (2003)). Similarly, RNA aptamers have been demonstrated to target the Ku DNA repair proteins with resulting sensitization of breast cancer cells to etoposide (Zhang et al, Int. J. Mol. Med. 74:153 (2004)).

By "small molecule" we mean a low molecular weight organic compound of 900 Daltons
15 or less. Although large biopolymers such as nucleic acids, proteins, and polysaccharides (such as starch or cellulose) are not included as "small molecules", their constituent monomers (ribo- or deoxyribonucleotides, amino acids, and monosaccharides, respectively) and oligomers (i.e. short polymers such as dinucleotides, peptides such as the antioxidant glutathione, and disaccharides such as
20 sucrose) are included. The production of small molecules is described in Mayes & Whitcombe, 2005, *Adv. Drug Deliv. Rev.* **57**:1742-78 and Root-Bernstein & Dillon, 2008, *Curr. Pharm. Des.* **14**:55-62.

CovX-Bodies are created by covalently joining a pharmacophore via a linker to the
25 binding site of a specially-designed antibody, effectively reprogramming the antibody (Tryder et al., 2007, *Bioorg. Med. Chem. Lett.*, 17:501-6). The result is a new class of chemical entities that is formed where each component contributes desirable traits to the intact *CovX-Body* – in particular, the entity has the biologic actions of the peptide and the extended half-life of the antibody.

30

It may be preferred that the binding moiety is in an isolated and/or purified form.

In an embodiment, it is preferred that the antibody or the binding moiety according to the invention does not specifically bind to human discoidin domain receptor 2 (DDR2),
35 especially the extracellular domain thereof. If the antibody or the binding moiety does bind to the extracellular domain of human DDR2, it is preferred that it does so with a

lower affinity than for the extracellular domain of DDR1, for example 10x, 100x or 1000x lower affinity than for DDR1, as described above.

The sequence of human DDR2, taken from UniProt Accession No. Q16832, is as follows:

```

      10      20      30      40      50      60
5  MILIPRMLLV LFLLLPILSS AKAQVNPAIC RYPLGMSGGQ IPDEDITASS QWSESTAAKY

      70      80      90     100     110     120
   GRLDSEEGDG AWCPEIPVEP DDLKEFLQID LHTLHFITLV GTQGRHAGGH GIEFAPMYKI

      130     140     150     160     170     180
10  NYSRDGTRWI SWRNRHGKQV LDGNSNPYDI FLKDLEPPIV ARFVRFIPVT DHSMNVCMRV

      190     200     210     220     230     240
   ELYGCVWLDG LVSYNAPAGQ QFVLPGGSII YLNDSVYDGA VGYSMTEGLG QLTGCVSGLD

      250     260     270     280     290     300
15  DFTQTHEYHV WPGYDYVGWR NESATNGYIE IMFEFDRIRN FTTMKVHCNN MFAKGVKIFK

      310     320     330     340     350     360
20  EVQCYFRSEA SEWEPNAISF PLVLDDVNPS ARFVTVPLHH RMASAIKCQY HFADTWMMFS

      370     380     390     400     410     420
   EITFQSDAAM YNNSEALPTS PMAPTTYDPM LKVDDSNTRI LIGCLVAIIF ILLAIIVIIL

      430     440     450     460     470     480
25  WRQFWQKMLE KASRRMLDDE MTVSLSLPSD SSMFNNNRSS SPSEQGSNST YDRIFPLRPD

      490     500     510     520     530     540
   YQEPSRLIRK LPEFAPGEEE SGCSGVVKPV QPSGPEGVPH YAEADIVNLQ GVTGGNTYSV

      550     560     570     580     590     600
30  PAVTMDLLSG KDVAVEEFPR KLLTFKEKLG EGQFGEVHLC EVEGMEKFKD KDFALDVSAN

      610     620     630     640     650     660
35  QPVLVAVKML RADANKNARN DFLKEIKIMS RLKDPNIIHL LAVCITDDPL CMITEYMENG

      670     680     690     700     710     720
   DLNQFLSRHE PPNSSSSDVR TVSYTNLKFM ATQIASGMKY LSSLNFEVHRD LATRNCLVGK

      730     740     750     760     770     780
40  NYTIKIADFG MSRNLYSGDY YRIQGRAVLP IRWMSWESIL LGKFTTASDV WAFGVTLWET

      790     800     810     820     830     840
   FTFCQEQPYS QLSDEQVIEN TGEFFRDQGR QTYLPQPAIC PDSVYKLMLS CWRRDTKNRP

45      850
   SFQEIHL LLL QQGDE

```

Methods for determining whether, and to what extent, an antibody or other moiety binds to a protein such as DDR2 are very well known in the art.

50

Another aspect of the invention provides a polynucleotide encoding an antibody or a binding moiety of the invention - provided that the binding moiety is a polypeptide.

The invention also includes an expression vector comprising the polynucleotide, and a host cell comprising the polynucleotide or the expression vector. Typically the host cell is capable of expressing producing the antibody or the polypeptide binding moiety, such that it can readily be obtained and purified, as is well known in the art.

5

Another aspect of the invention provides a compound comprising an antibody or a binding moiety as defined above, and a detectable moiety.

By a "detectable moiety" we include the meaning that the moiety is one which, when located at the target site following administration of an agent of the invention to a patient, may be detected, typically non-invasively from outside the body and the site of the target located. The detectable moiety may be a single atom or molecule which is either directly or indirectly involved in the production of a detectable species. Thus, the agents of this embodiment of the invention are useful in imaging and diagnosis, especially of cells and tissues that express DDR1. Detectable compounds also aid in research and development.

Suitable detectable moieties are well known in medicinal chemistry and the linking of these moieties to polypeptides and proteins is well known in the art. Examples of detectable moieties include, but are not limited to, the following: radioisotopes (e.g. ^3H , ^{14}C , ^{35}S , ^{123}I , ^{125}I , ^{131}I , ^{99}Tc , ^{111}In , ^{90}Y , ^{188}Re), radionuclides (e.g. ^{11}C , ^{18}F , ^{64}Cu), fluorescent labels (e.g. FITC, rhodamine, lanthanide phosphors, carbocyanine), enzymatic labels (e.g. horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups and predetermined polypeptide epitopes recognised by a secondary reporter (e.g. leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

Preferably, the detectable moiety comprises a radioactive atom, such as a radioactive atom selected from the group consisting of: technetium-99; technetium-99m; iodine-123; iodine-124; iodine-131; indium-111; fluorine-18; fluorine-19; carbon-11; carbon-13; copper-64; nitrogen-13; nitrogen-15; oxygen-15; oxygen-17; arsenic-72; gadolinium; manganese; iron; deuterium; tritium; yttrium-86; zirconium-89.

35

The radio- or other labels may be incorporated into the agents of the invention in known ways. For example, if the binding moiety is a polypeptide it may be biosynthesised or

may be synthesised by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as ^{99m}Tc , ^{123}I , ^{186}Rh , ^{188}Rh and ^{111}In can, for example, be attached *via* cysteine residues in the binding moiety. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker *et al* (1978) *Biochem. Biophys. Res. Comm.* **80**, 49-57, which is
5 incorporated herein by reference) can be used to incorporate ^{123}I . Reference ("Monoclonal Antibodies in Immunoscintigraphy", J-F Chatal, CRC Press, 1989, which is incorporated herein by reference) describes other methods in detail.

10 Another aspect of the invention provides a compound comprising an antibody or a binding moiety as defined above, and a cytotoxic moiety. Such a compound may be used in the treatment and medical use aspects of the invention described below.

By a "cytotoxic moiety" we include the meaning that the moiety is one which is capable of
15 inducing cell death *in vivo* or *in vitro*, for example when administered to a patient. The cytotoxic moiety may be a single atom or molecule which is either directly or indirectly involved in inducing cell death. Thus, the agents of this embodiment of the invention are useful in therapy (for example, where it is desired to remove or destroy one or more cell in an individual).

20 Suitable cytotoxic moieties are well known in medicinal chemistry and the linking of these moieties to polypeptides and proteins is well known in the art. For example, when each moiety of the agent of the invention is a polypeptide, the two portions may be linked together by any of the conventional ways of cross-linking polypeptides, such as those
25 generally described in O'Sullivan *et al* (1979) *Anal. Biochem.* **100**, 100-108. For example, the binding moiety may be enriched with thiol groups and the further moiety reacted with a bifunctional agent capable of reacting with those thiol groups, for example the N-hydroxysuccinimide ester of iodoacetic acid (NHIA) or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP). Amide and thioether bonds, for example achieved with
30 m-maleimidobenzoyl-N-hydroxysuccinimide ester, are generally more stable *in vivo* than disulphide bonds.

One avenue towards the development of more selective, and thus better, anticancer drugs is the targeted delivery of bioactive molecules to the tumour environment by
35 means of binding molecules (for example, human antibodies) that are specific for tumour endothelial markers. Due to their accessibility and to the therapeutic options that they allow (for example, intraluminal blood coagulation or recruitment of immune cells),

markers selectively expressed on tumour cells are ideally suited for ligand-based tumour-targeting strategies, allowing for the targeting cytotoxic agents directly to the tumour. Thus these compounds may be useful in targeting a cytotoxic agent to tumour/cells expressing DDR1 in the body of an individual.

5

Typically the cytotoxic moiety is selected from a directly cytotoxic chemotherapeutic agent, a directly cytotoxic polypeptide, a moiety which is able to convert a prodrug into a cytotoxic drug, a radiosensitizer, a directly cytotoxic nucleic acid, a nucleic acid molecule that encodes a directly or indirectly cytotoxic polypeptide or a radioactive atom.

10 Examples of such cytotoxic moieties, as well as methods of making the conjugates comprising the antibody and the cytotoxic moiety, are provided in WO 02/36771, incorporated herein by reference.

The cytotoxic moiety may be directly or indirectly toxic to cells expressing DDR1. By
15 "directly cytotoxic" we include the meaning that the moiety is one which on its own is cytotoxic. By "indirectly cytotoxic" we include the meaning that the moiety is one which, although is not itself cytotoxic, can induce cytotoxicity, for example by its action on a further molecule or by further action on it.

20 In one embodiment the cytotoxic moiety is a cytotoxic chemotherapeutic agent. Cytotoxic chemotherapeutic agents are well known in the art. Cytotoxic chemotherapeutic agents, such as anticancer agents, include those listed herein.

Various of these cytotoxic moieties, such as cytotoxic chemotherapeutic agents, have
25 previously been attached to antibodies and other targeting agents, and so compounds of the invention comprising these agents may readily be made by the person skilled in the art. For example, carbodiimide conjugation (Bauminger & Wilchek (1980) *Methods Enzymol.* **70**, 151-159) may be used to conjugate a variety of agents, including doxorubicin, to antibodies. Other methods for conjugating a cytotoxic moiety to an
30 antibody can also be used. For example, sodium periodate oxidation followed by reductive alkylation of appropriate reactants can be used, as can glutaraldehyde cross-linking. Methods of cross-linking polypeptides are known in the art and described in WO 2004/046191. However, it is recognised that, regardless of which method of producing a compound of the invention is selected, a determination must be made that
35 the antibody maintains its targeting ability and that the attached moiety maintains its relevant function.

In a further embodiment of the invention, the cytotoxic moiety may be a cytotoxic peptide or polypeptide moiety by which we include any moiety which leads to cell death. Cytotoxic peptide and polypeptide moieties are well known in the art and include, for example, ricin, abrin, *Pseudomonas* exotoxin, tissue factor and the like. Methods for linking them to targeting moieties such as antibodies are also known in the art. The use of ricin as a cytotoxic agent is described in Burrows & Thorpe (1993) *Proc. Natl. Acad. Sci. USA* **90**, 8996-9000, and the use of tissue factor, which leads to localised blood clotting and infarction of a tumour, has been described by Ran *et al* (1998) *Cancer Res.* **58**, 4646-4653 and Huang *et al* (1997) *Science* **275**, 547-550. Tsai *et al* (1995) *Dis. Colon Rectum* **38**, 1067-1074 describes the abrin A chain conjugated to a monoclonal antibody. Other ribosome inactivating proteins are described as cytotoxic agents in WO 96/06641. *Pseudomonas* exotoxin may also be used as the cytotoxic polypeptide moiety (Aiello *et al* (1995) *Proc. Natl. Acad. Sci. USA* **92**, 10457-10461).

Certain cytokines, such as TNF α , INF γ and IL-2, may also be useful as cytotoxic agents.

Certain radioactive atoms may also be cytotoxic if delivered in sufficient doses. Thus, the cytotoxic moiety may comprise a radioactive atom which, in use, delivers a sufficient quantity of radioactivity to the target site so as to be cytotoxic. Suitable radioactive atoms include phosphorus-32, iodine-125, iodine-131, indium-111, rhenium-186, rhenium-188 or yttrium-90, or any other isotope which emits enough energy to destroy neighbouring cells, organelles or nucleic acid. Preferably, the isotopes and density of radioactive atoms in the compound of the invention are such that a dose of more than 4000 cGy (preferably at least 6000, 8000 or 10000 cGy) is delivered to the target site and, preferably, to the cells at the target site and their organelles, particularly the nucleus.

The radioactive atom may be attached to the antibody in known ways. For example EDTA or another chelating agent may be attached to the antibody and used to attach ^{111}In or ^{90}Y . Tyrosine residues may be labelled with ^{125}I or ^{131}I .

The cytotoxic moiety may be a radiosensitizer. Radiosensitizers include fluoropyrimidines, thymidine analogues, hydroxyurea, gemcitabine, fludarabine, nicotinamide, halogenated pyrimidines, 3-aminobenzamide, 3-aminobenzodiamide, etanixadole, pimonidazole and misonidazole (see, for example, McGinn *et al* (1996) *J. Natl. Cancer Inst.* **88**, 1193-11203; Shewach & Lawrence (1996) *Invest. New Drugs* **14**, 257-263; Horsman (1995) *Acta Oncol.* **34**, 571-587; Shenoy & Singh (1992) *Clin. Invest.*

10, 533-551; Mitchell *et al* (1989) *Int. J. Radiat. Biol.* **56**, 827-836; Iliakis & Kurtzman (1989) *Int. J. Radiat. Oncol. Biol. Phys.* **16**, 1235-1241; Brown (1989) *Int. J. Radiat. Oncol. Biol. Phys.* **16**, 987-993; Brown (1985) *Cancer* **55**, 2222-2228).

- 5 The cytotoxic moiety may be a procoagulant factor, such as the extracellular domain of tissue factor (Rippmann *et al* (2000) "Fusion of the tissue factor extracellular domain to a tumour stroma specific single-chain fragment variable antibody results in an antigen-specific coagulation-promoting molecule." *Biochem J.* 349: 805-12; Huang *et al* (1997) "Tumor infarction in mice by antibody-directed targeting of tissue factor to tumor vasculature." *Science*. **275**(5299): 547-550.

- The cytotoxic moiety may be an indirectly cytotoxic polypeptide. In a particularly preferred embodiment, the indirectly cytotoxic polypeptide is a polypeptide which has enzymatic activity and can convert a relatively non-toxic prodrug into a cytotoxic drug.
- 15 When the targeting moiety is an antibody this type of system is often referred to as ADEPT (Antibody-Directed Enzyme Prodrug Therapy). The system requires that the targeting moiety locates the enzymatic portion to the desired site in the body of the patient (e.g. the site of new vascular tissue associated with a tumour) and after allowing time for the enzyme to localise at the site, administering a prodrug which is a substrate
- 20 for the enzyme, the end product of the catalysis being a cytotoxic compound. The object of the approach is to maximise the concentration of drug at the desired site and to minimise the concentration of drug in normal tissues (Senter *et al* (1988) "Anti-tumor effects of antibody-alkaline phosphatase conjugates in combination with etoposide phosphate" *Proc. Natl. Acad. Sci. USA* **85**, 4842-4846; Bagshawe (1987) *Br. J. Cancer* **56**, 531-2; and Bagshawe, *et al* (1988) "A cytotoxic agent can be generated selectively at cancer sites" *Br. J. Cancer*. **58**, 700-703); Bagshawe (1995) *Drug Dev. Res.* **34**, 220-230 and WO 2004/046191, describe various enzyme/prodrug combinations which may be suitable in the context of this invention.

- 30 Typically, the prodrug is relatively non-toxic compared to the cytotoxic drug. Typically, it has less than 10% of the toxicity, preferably less than 1% of the toxicity as measured in a suitable *in vitro* cytotoxicity test.

- It is likely that the moiety which is able to convert a prodrug to a cytotoxic drug will be
- 35 active in isolation from the rest of the compound but it is necessary only for it to be active when (a) it is in combination with the rest of the compound and (b) the compound is attached to, adjacent to or internalised in target cells.

The further moiety may be one which becomes cytotoxic, or releases a cytotoxic moiety, upon irradiation. For example, the boron-10 isotope, when appropriately irradiated, releases α particles which are cytotoxic (US 4,348,376; Primus *et al* (1996) *Bioconj. Chem.* **7**: 532-535).

Similarly, the cytotoxic moiety may be one which is useful in photodynamic therapy such as photofrin (see, for example, Dougherty *et al* (1998) *J. Natl. Cancer Inst.* **90**, 889-905).

Preferably, the invention provides a compound comprising an antibody or binding moiety as described above, and a drug to be delivered to a cell having a DDR1 receptor localised on its surface, for example a cancer or tumour cell, including tumour stem cells.

In an embodiment, the invention provides a compound wherein the drug comprises a immunosuppressive drug, such as an anti-inflammatory drug. Such drugs are known to those in the arts of medicine and pharmacology. The immunosuppressive drug may be selected from the group comprising or consisting of: a glucocorticoid; methotrexate; cyclophosphamide; 6-mercaptopurin; cyclosporine; tacrolimus; mycophenolate mofetil; sirolimus; everolimus; an siRNA molecule capable of inhibiting synthesis of proinflammatory cytokines (such as TNF); a non-steroidal anti-inflammatory drug (NSAIDs, such as aspirin, ibuprofen); a steroid (such as vitamin D); a disease-modifying anti-rheumatic drug (DMARDs, such as penicillamin, sulfasalazin, cyclosporine). Exemplary glucocorticoids may be selected from the group comprising or consisting of: cortisone and derivatives thereof (such as hydrocortisone); prednisone and derivatives thereof (such as prednisolone, methylprednisolone, methylprednisolone-acetate, methylprednisolone-succinate); dexamethasone and derivatives thereof; triamcinolone and derivatives thereof (such as triamcinolonehexacetonuid, triamcinolonacetonamid); paramethasone; betamethasone; fluhydrocortisone; fluocinolone. Such a compound may be useful, for example, in the treatment of an inflammatory condition or disorder.

By 'treatment' we include both therapeutic and prophylactic treatment of a subject/patient. The term 'prophylactic' is used to encompass the use of an agent, medicament or pharmaceutical formulation described herein which either prevents or reduces the likelihood of a condition or disorder in the patient.

It is appreciated that for the prevention or treatment of a condition or disorder, the appropriate dosage of an agent will depend on the type of condition or disorder to be

treated, the severity and of course of the condition or disorder, whether the agent is administered for prophylactic or therapeutic purposes, the course of previous therapy and the patient's clinical history and response to the agent. According to a further embodiment of the invention, the effectiveness of an agent of the invention in alleviating the symptoms, preventing or treating a condition or disorder may be improved by serial administering or administration in combination with another agent that is effective for the same condition or disorder, such as conventional therapeutic agents known for the intended therapeutic indication.

10 A further aspect of the invention provides a pharmaceutical composition comprising an antibody or a binding moiety or a compound of the invention as described herein and a pharmaceutically acceptable diluent, carrier or excipient.

As used herein, 'pharmaceutical composition' means a therapeutically effective formulation according to the invention.

A 'therapeutically effective amount', or 'effective amount', or 'therapeutically effective', as used herein, refers to that amount which provides a therapeutic effect for a given condition and administration regimen. This is a predetermined quantity of active material calculated to produce a desired therapeutic effect in association with the required additive and diluent, *i.e.* a carrier or administration vehicle. Further, it is intended to mean an amount sufficient to reduce or prevent a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in a host.

25 As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluent, carrier or excipient.

30 In the methods and use for manufacture of compositions of the invention, a therapeutically effective amount of the active component is provided. A therapeutically effective amount can be determined by the ordinary skilled medical worker based on patient characteristics, such as age, weight, sex, condition, complications, other diseases, *etc.*, as is well known in the art.

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In the case of cancer, the therapeutically effective amount of the drug has a therapeutic effect and as such can reduce the number of cancer cells; decrease tumorigenicity,

tumorigenic frequency or tumorigenic capacity; reduce the number or frequency of cancer stem cells; reduce the tumor size; inhibit or stop cancer cell infiltration into peripheral organs including, for example, the spread of cancer into soft tissue and bone; inhibit and stop tumor metastasis; inhibit and stop tumor growth; relieve to some extent
5 one or more of the symptoms associated with the cancer; reduce morbidity and mortality; improve quality of life; or a combination of such effects.

By "pharmaceutically acceptable" is included that the formulation is sterile and pyrogen free. Suitable pharmaceutical carriers, diluents and excipients are well known in the art
10 of pharmacy. The carrier(s) must be "acceptable" in the sense of being compatible with the compound and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free; however, other acceptable carriers may be used.

15 In an embodiment, the pharmaceutical compositions or formulations of the invention are formulated for parenteral administration, more particularly for intravenous administration. In a preferred embodiment, the pharmaceutical composition is suitable for intravenous administration to a patient, for example by injection.

20 Thus, typically and preferably, the composition is administered intravenously or by intraperitoneal administration to the patient.

Suitably the compound is administered as an infusion or as a bolus injection.

25 Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

30

In an alternative preferred embodiment, the pharmaceutical composition is suitable for topical administration to a patient.

Preferably, the formulation is a unit dosage containing a daily dose or unit, daily sub-dose
35 or an appropriate fraction thereof, of the active ingredient.

The antibodies, agents, medicaments and pharmaceutical compositions may be administered orally or by any parenteral route, in the form of a pharmaceutical formulation comprising the active ingredient, optionally in the form of a non-toxic organic, or inorganic, acid, or base, addition salt, in a pharmaceutically acceptable dosage form.

5 Depending upon the disorder and patient to be treated, as well as the route of administration, the compositions may be administered at varying doses.

The antibodies, agents, medicaments and pharmaceutical compositions of the invention may be delivered using an injectable sustained-release drug delivery system. These are
10 designed specifically to reduce the frequency of injections. An example of such a system is Nutropin Depot which encapsulates recombinant human growth hormone (rhGH) in biodegradable microspheres that, once injected, release rhGH slowly over a sustained period. Preferably, delivery is performed intra-muscularly (i.m.) and/or sub-cutaneously (s.c.) and/or intravenously (i.v.).

15 The antibodies, agents, medicaments and pharmaceutical compositions of the invention can be administered by a surgically implanted device that releases the drug directly to the required site. For example, Vitrasert releases ganciclovir directly into the eye to treat CMV retinitis. The direct application of this toxic agent to the site of disease achieves
20 effective therapy without the drug's significant systemic side-effects.

Electroporation therapy (EPT) systems can also be employed for the administration of the agents, medicaments and pharmaceutical compositions of the invention. A device which delivers a pulsed electric field to cells increases the permeability of the cell
25 membranes to the drug, resulting in a significant enhancement of intracellular drug delivery.

The agents, medicaments and pharmaceutical compositions of the invention can also be delivered by electro-incorporation (EI). EI occurs when small particles of up to 30
30 microns in diameter on the surface of the skin experience electrical pulses identical or similar to those used in electroporation. In EI, these particles are driven through the stratum corneum and into deeper layers of the skin. The particles can be loaded or coated with drugs or genes or can simply act as "bullets" that generate pores in the skin through which the drugs can enter.

35 An alternative method of delivery of the antibodies, agents, medicaments and pharmaceutical compositions of the invention is the ReGel injectable system that is

thermo-sensitive. Below body temperature, ReGel is an injectable liquid while at body temperature it immediately forms a gel reservoir that slowly erodes and dissolves into known, safe, biodegradable polymers. The active substance is delivered over time as the biopolymers dissolve.

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The antibodies, agents, medicaments and pharmaceutical compositions of the invention can also be delivered orally. The process employs a natural process for oral uptake of vitamin B₁₂ and/or vitamin D in the body to co-deliver proteins and peptides. By riding the vitamin B₁₂ and/or vitamin D uptake system, the agents, medicaments and pharmaceutical compositions of the invention can move through the intestinal wall. Complexes are synthesised between vitamin B₁₂ analogues and/or vitamin D analogues and the drug that retain both significant affinity for intrinsic factor (IF) in the vitamin B₁₂ portion/vitamin D portion of the complex and significant bioactivity of the active substance of the complex.

15

Preferably, the medicaments and/or pharmaceutical compositions of the present invention is a unit dosage containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of the active ingredient.

20 The antibodies, agents, medicaments and pharmaceutical compositions of the invention will normally be administered orally or by any parenteral route, in the form of a pharmaceutical composition comprising the active ingredient, optionally in the form of a non-toxic organic, or inorganic, acid, or base, addition salt, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated, as well as the route of administration, the compositions may be administered at varying doses.

25

In human therapy, the antibodies, agents, medicaments and pharmaceutical compositions of the invention can be administered alone but will generally be administered in admixture with a suitable pharmaceutical excipient, diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

30

For example, the antibodies, agents, medicaments and pharmaceutical compositions of the invention can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed- or controlled-release applications. The

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agents, medicaments and pharmaceutical compositions of the invention may also be administered via intracavernosal injection.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxy-propylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the agents, medicaments and pharmaceutical compositions of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

The antibodies, agents, medicaments and pharmaceutical compositions of the invention can be administered parenterally, for example, intravenously, intra-arterially, intraperitoneally, intra-theccally, intraventricularly, intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

Medicaments and pharmaceutical compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The medicaments and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised)

condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

5

For oral and parenteral administration to human patients, the daily dosage level of the agents, medicaments and pharmaceutical compositions of the invention will usually be from 0.002 to 0.4 mg/kg and/or 0.1 mg/kg to 20mg/kg administered in single or divided doses.

10

Thus, for example, the tablets or capsules of the medicaments and pharmaceutical compositions of the invention may contain from 5mg to 1400mg (for example, from 7mg to 1400mg, or 5mg to 1000mg) and may preferably contain 5mg to 200mg of active agent for administration singly or two or more at a time, as appropriate.

15

In one embodiment, the agents, medicaments and pharmaceutical compositions of the invention are administered at a dosage ranging from 0.02mg/kg to 2mg/kg and at a frequency ranging from twice per week to once per month.

20 The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention.

25

The antibodies, agents, medicaments and pharmaceutical compositions of the invention can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, e.g. 30 dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoro-ethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A3 or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA3), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray or nebuliser may contain a 35 solution or suspension of the active agent, e.g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler

or insufflator may be formulated to contain a powder mix of an agent of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains 5mg to 1400mg (for example, from 7mg to 1400mg, or 5mg to 1000mg) and preferably contain 5mg to 200mg of an agent of the invention for delivery to the patient. It will be appreciated that the overall daily dose with an aerosol will vary from patient to patient, and may be administered in a single dose or, more usually, in divided doses throughout the day.

Alternatively, the antibodies, agents, medicaments and pharmaceutical compositions of the invention can be administered in the form of a suppository or pessary, or they may be applied topically in the form of a lotion, solution, cream, gel, ointment or dusting powder. The agents, medicaments and pharmaceutical compositions of the invention may also be transdermally administered, for example, by the use of a skin patch. They may also be administered by the ocular route, particularly for treating diseases of the eye.

For ophthalmic use, the agents, medicaments and pharmaceutical compositions of the invention can be formulated as micronised suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For application topically to the skin, the antibodies, agents, medicaments and pharmaceutical compositions of the invention can be formulated as a suitable ointment containing the active agent suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene agent, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or

sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

Generally, in humans, oral or parenteral administration of the agents, medicaments and pharmaceutical compositions of the invention is the preferred route, being the most convenient.

The agents of the invention may be formulated at various concentrations, depending on the efficacy/toxicity of the compound being used, for example as described in the accompanying Examples. For *in vitro* applications, formulations may comprise a lower concentration of a compound of the invention.

Thus, the present invention provides a pharmaceutical formulation comprising an amount of an antibody or antigen-binding fragment, or fusion or derivative thereof, of the invention effective to treat various conditions (as described herein).

Preferably, the pharmaceutical composition is adapted for delivery by a route selected from the group comprising: intravenous; intramuscular; subcutaneous; intra-articular; pulmonary; intranasal; intraocular; intrathecal.

The present invention also includes pharmaceutical compositions comprising pharmaceutically acceptable acid or base addition salts of the polypeptide binding moieties of the present invention. The acids which are used to prepare the pharmaceutically acceptable acid addition salts of the aforementioned base compounds useful in this invention are those which form non-toxic acid addition salts, *i.e.* salts containing pharmacologically acceptable anions, such as the hydrochloride, hydrobromide, hydroiodide, nitrate, sulphate, bisulphate, phosphate, acid phosphate, acetate, lactate, citrate, acid citrate, tartrate, bitartrate, succinate, maleate, fumarate, gluconate, saccharate, benzoate, methanesulphonate, ethanesulphonate, benzenesulphonate, *p*-toluenesulphonate and pamoate [*i.e.* 1,1'-methylene-bis-(2-hydroxy-3 naphthoate)] salts, among others.

Pharmaceutically acceptable base addition salts may also be used to produce pharmaceutically acceptable salt forms of the agents according to the present invention.

The chemical bases that may be used as reagents to prepare pharmaceutically acceptable base salts of the present agents that are acidic in nature are those that form

non-toxic base salts with such compounds. Such non-toxic base salts include, but are not limited to those derived from such pharmacologically acceptable cations such as alkali metal cations (e.g. potassium and sodium) and alkaline earth metal cations (e.g. calcium and magnesium), ammonium or water-soluble amine addition salts such as N-methylglucamine-(meglumine), and the lower alkanolammonium and other base salts of pharmaceutically acceptable organic amines, among others.

The antibodies, agents and/or polypeptide binding moieties of the invention may be lyophilised for storage and reconstituted in a suitable carrier prior to use. Any suitable lyophilisation method (e.g. spray drying, cake drying) and/or reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilisation and reconstitution can lead to varying degrees of antibody activity loss (e.g. with conventional immunoglobulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted upward to compensate. In one embodiment, the lyophilised (freeze dried) polypeptide binding moiety loses no more than about 20%, or no more than about 25%, or no more than about 30%, or no more than about 35%, or no more than about 40%, or no more than about 45%, or no more than about 50% of its activity (prior to lyophilisation) when re-hydrated.

In a further aspect, the invention provides a kit comprising an agent or a pharmaceutical composition as defined herein. Thus, there may be provided a kit for use in the therapeutic treatment of the conditions defined herein.

Alternatively, the kit may comprise a detectable antibody or antigen-binding fragment or derivative thereof according to the invention, suitable for use in diagnosis. Such a diagnostic kit may comprise, in an amount sufficient for at least one assay, the diagnostic agent as a separately packaged reagent. Instructions for use of the packaged reagent are also typically included. Such instructions typically include a tangible expression describing reagent concentrations and/or at least one assay method parameter such as the relative amounts of reagent and sample to be mixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions and the like.

In a further aspect, the invention provides antibodies, agents, binding moieties or compounds of the invention for use in medicine. Methods of manufacturing a medicament using an active agent, such as the agent of the invention, are well known to persons skilled in the art of medicine and pharmacy.

In an embodiment, the pharmaceutical composition may further comprise at least one additional anti-cancer agent.

The additional anticancer agent may be selected from alkylating agents including nitrogen
 5 mustards such as mechlorethamine (HN₂), cyclophosphamide, ifosfamide, melphalan (L-sarcolysin) and chlorambucil; ethylenimines and methylmelamines such as hexamethylmelamine, thiotepa; alkyl sulphonates such as busulphan; nitrosoureas such as carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU) and streptozocin (streptozotocin); and triazenes such as decarbazine (DTIC; dimethyltriazenoimidazole-
 10 carboxamide); antimetabolites including folic acid analogues such as methotrexate (amethopterin); pyrimidine analogues such as fluorouracil (5-fluorouracil; 5-FU), floxuridine (fluorodeoxyuridine; FdR) and cytarabine (cytosine arabinoside); and purine analogues and related inhibitors such as mercaptopurine (6-mercaptopurine; 6-MP), thioguanine (6-thioguanine; TG) and pentostatin (2'-deoxycofomycin); natural products including vinca
 15 alkaloids such as vinblastine (VLB) and vincristine; epipodophyllotoxins such as etoposide and teniposide; antibiotics such as dactinomycin (actinomycin D), daunorubicin (daunomycin; rubidomycin), doxorubicin, bleomycin, plicamycin (mithramycin) and mitomycin (mitomycin C); enzymes such as L-asparaginase; and biological response modifiers such as interferon alphenomes; miscellaneous agents including platinum
 20 coordination complexes such as cisplatin (*cis*-DDP) and carboplatin; anthracenedione such as mitoxantrone and anthracycline; substituted urea such as hydroxyurea; methyl hydrazine derivative such as procarbazine (N-methylhydrazine, MIH); and adrenocortical suppressant such as mitotane (*o,p'*-DDD) and aminoglutethimide; taxol and analogues/derivatives; cell
 25 cycle inhibitors; proteosome inhibitors such as Bortezomib (Velcade®); signal transductase (e.g. tyrosine kinase) inhibitors such as Imatinib (Glivec®), COX-2 inhibitors, and hormone agonists/antagonists such as flutamide and tamoxifen.

The clinically used anticancer agents are typically grouped by mechanism of action: Alkylating agents, Topoisomerase I inhibitors, Topoisomerase II inhibitors, RNA/DNA
 30 antimetabolites, DNA antimetabolites and Antimitotic agents. The US NIH/National Cancer Institute website lists 122 compounds (http://dtp.nci.nih.gov/docs/cancer/searches/standard_mechanism.html), all of which may be used in conjunction with the compound. They include Alkylating agents including Asaley, AZQ, BCNU, Busulfan, carboxyphthalatoplatinum, CBDCA, CCNU, CHIP, chlorambucil, chlorozotocin, *cis*-
 35 platinum, clomesone, cyanomorpholino-doxorubicin, cyclodisone, dianhydrogalactitol, fluorodopan, hepsulfam, hycanthone, melphalan, methyl CCNU, mitomycin C, mitozolamide, nitrogen mustard, PCNU, piperazine, piperazinedione, pipobroman,

porfiromycin, spirohydantoin mustard, teroxirone, tetraplatin, thio-tepa, triethylenemelamine, uracil nitrogen mustard, Yoshi-864; anitmitotic agents including allocolchicine, Halichondrin B, colchicine, colchicine derivative, dolastatin 10, maytansine, rhizoxin, taxol, taxol derivative, thiocolchicine, trityl cysteine, vinblastine sulphate, vincristine sulphate; Topoisomerase I Inhibitors including camptothecin, camptothecin, Na salt, aminocamptothecin, 20 camptothecin derivatives, morpholinodoxorubicin; Topoisomerase II Inhibitors including doxorubicin, amonafide, m-AMSA, anthrapyrazole derivative, pyrazoloacridine, bisantrene HCL, daunorubicin, deoxydoxorubicin, mitoxantrone, menogaril, N,N-dibenzyl daunomycin, oxanthrazole, rubidazole, VM-26, VP-16; RNA/DNA antimetabolites including L-alanosine, 5-azacytidine, 5-fluorouracil, acivicin, 3 aminopterin derivatives, an antifol, Baker's soluble antifol, dichlorallyl lawsone, brequinar, florafur (pro-drug), 5,6-dihydro-5-azacytidine, methotrexate, methotrexate derivative, N-(phosphonoacetyl)-L-aspartate (PALA), pyrazofurin, trimetrexate; DNA antimetabolites including, 3-HP, 2'-deoxy-5-fluorouridine, 5-HP, alpha-TGDR, aphidicolin glycinate, ara-C, 5-aza-2'-deoxycytidine, beta-TGDR, cyclocytidine, guanazole, hydroxyurea, inosine glycodialdehyde, macbecin II, pyrazoloimidazole, thioguanine and thiopurine.

It may be preferred that the at least one additional anticancer agent is selected from cisplatin, carboplatin, 5-fluorouracil, paclitaxel, mitomycin C, doxorubicin, gemcitabine, tomudex, pemetrexed, methotrexate, irinotecan, oxaliplatin, or combinations thereof.

It may also be preferred that the further anticancer agent is a platinum-based chemotherapeutic agent. Clinically approved platinum-based chemotherapeutic agents include carboplatin (*cis*-diammine(cyclobutane-1,1-dicarboxylate-*O,O'*)platinum(II); CAS Registry Number 41575-94-4), cisplatin ((*SP*-4-2)-diamminedichloridoplatinum; CAS Registry number 15663-271), and oxaliplatin ([*(1R,2R)*-cyclohexane-1,2-diamine](ethanedioate-*O,O'*)platinum(II); CAS Registry Number 63121-00-6). Oxaliplatin may also be typically administered with fluorouracil and leucovorin in a combination known as FOLFOX. These platinum-based chemotherapeutic agents are typically administered intravenously as a short-term infusion in physiological saline, as is well known in the art.

When the additional anticancer agent or combination of agents has been shown to be particularly effective for a specific tumour type, it may be preferred that the antibody, agent, binding moiety or compound of the invention is used in combination with that further anticancer agent(s) to treat that specific tumour type.

Another aspect of the invention provides a kit of parts comprising an antibody, binding moiety or compound of the invention and at least one additional anti-cancer agent.

- 5 Yet another aspect of the invention provides an antibody, binding moiety or compound of the invention and, optionally, at least one additional anti-cancer agent, for use in medicine.

10 Still another aspect of the invention provides method of inhibiting activity of DDR1 on a cell, the method comprising contacting the cell with an effective amount of an antibody, binding moiety or compound of the invention. In an embodiment, the cell is a tumour cell, for example a tumour stem cell. This inhibition may be *in vivo* or *in vitro*. The tumour cell may be, for example, a breast, colorectal, hepatic, renal, lung, pancreatic, ovarian, prostate or head and neck tumour cell.

15 Still a yet further aspect of the invention provides method of inhibiting growth of a tumour in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody, binding moiety, compound or pharmaceutical composition of the invention, and, optionally, at least one additional anti-cancer agent.

20 The stem cell nature of cancer is described in paragraphs [0004], [0005], [0054] and [0062] of WO 2010/019702. The identification of DDR1 as a cancer stem cell marker, and the use of anti-DDR1 antibodies to treat cancers comprising cancer stem cells, is further described in paragraphs [0063] to [0065], [0067], [0073] to [0075] and Examples
25 10-12 of WO 2010/019702. Each of these sections of WO 2010/019702 is incorporated herein in its entirety.

30 Accordingly, another aspect of the invention provides a method of reducing tumorigenicity of a tumour that comprises cancer stem cells in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody, binding moiety, compound or pharmaceutical composition of the invention, wherein the frequency of cancer stem cells in the tumour is reduced by the administration of the antibody binding moiety, compound or pharmaceutical composition of the invention.

35 Another aspect of the invention provides a method of treating cancer in a human patient, the method comprising administering to the patient a therapeutically effective amount of

an antibody, binding moiety, compound or pharmaceutical composition of the invention, and, optionally, at least one additional anti-cancer agent.

5 In these aspects, the tumour/cancer may be a breast, colorectal, hepatic, kidney, liver lung, pancreatic, gastrointestinal, ovarian, cervical, prostate, bladder, skin, endometrial, stomach, testis, thyroid, brain or head and neck tumour/cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, and nasal NK/T-cell lymphoma.

10 Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to both therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and prophylactic or preventative measures that prevent and/or slow the development of a targeted pathologic condition or disorder. Thus those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to
15 be prevented. In certain embodiments, a subject is successfully "treated" according to the methods of the present invention if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including, for example, the spread of cancer into soft tissue and bone; inhibition of or an absence of
20 tumor metastasis; inhibition or an absence of tumor growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; improvement in quality of life; reduction in tumorigenicity; reduction in the number or frequency of cancer stem cells; or some combination of effects.

25 The invention also includes an antibody, binding moiety, compound or pharmaceutical composition of the invention, and, optionally, at least one additional anti-cancer agent, for use in inhibiting growth of a tumour in a human patient, or for use in reducing the tumorigenicity of a tumour that comprises cancer stem cells in a human patient, or for use in treating cancer in a human patient.

30

The invention also includes the use of an antibody, binding moiety or compound of the invention, and, optionally, at least one additional anti-cancer agent, in the preparation of a medicament for inhibiting growth of a tumour in a human patient, or for use in reducing the tumorigenicity of a tumour that comprises cancer stem cells in a human patient, or for
35 use in treating cancer in a human patient.

The invention also includes the use of an antibody binding moiety or compound of the invention, in the preparation of a medicament for inhibiting growth of a tumour in a human patient, or for use in reducing the tumorigenicity of a tumour that comprises cancer stem cells in a human patient, or for use in treating cancer in a human patient, wherein the patient is one who is being administered a at least one additional anti-cancer agent.

Thus, in certain embodiments of these treatment aspects of the invention, the invention may further comprise administering to the patient at least one additional anticancer agent. The method may comprise administering to the individual a pharmaceutical composition containing the antibody, binding moiety or compound of the invention, and the further anticancer agent. However, it is appreciated that the antibody, binding moiety or compound of the invention and the additional anticancer agent may be administered separately, for instance by separate routes of administration. Thus it is appreciated that the antibody, binding moiety or compound of the invention and the at least one additional anticancer agent can be administered sequentially or (substantially) simultaneously. They may be administered within the same pharmaceutical formulation or medicament or they may be formulated and administered separately.

In an embodiment of the medical uses, the medicament containing the antibody, binding moiety or compound may also comprise the at least one additional anticancer agent.

In another embodiment of the medical uses, the individual to be treated may be one who is administered at least one additional anticancer agent. It is appreciated that the individual may be administered the additional anticancer agent at the same time as the medicament containing antibody, binding moiety or compound, although the individual may have been (or will be) administered the additional anticancer agent before (or after) receiving the medicament containing the compound.

Preferences for the additional anticancer agent are discussed above.

Another aspect of the invention provides a method of treating an inflammatory disorder, a fibrotic disorder or atherosclerosis in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody, binding moiety or pharmaceutical composition of the invention, and, optionally, at least one additional agent suitable for treating the said inflammatory disorder, a fibrotic disorder or atherosclerosis.

The invention includes an antibody, binding moiety or pharmaceutical composition of the invention for use in for use in treating an inflammatory disorder, a fibrotic disorder or atherosclerosis in a human patient, optionally, together with at least one additional agent
5 suitable for treating the inflammatory disorder, fibrotic disorder or atherosclerosis.

The invention also includes the use of an antibody or binding moiety of the invention in the preparation of a medicament for treating an inflammatory disorder, a fibrotic disorder or atherosclerosis in a human patient, wherein, optionally, the patient is one who is
10 administered at least one additional agent suitable for treating the inflammatory disorder, fibrotic disorder or atherosclerosis.

The invention also includes the use of an antibody or binding moiety of the invention, and at least one additional agent suitable for treating an inflammatory disorder, fibrotic
15 disorder or atherosclerosis, in the preparation of a medicament for treating an inflammatory disorder, a fibrotic disorder or atherosclerosis in a human patient.

In an embodiment, the inflammatory disorder may be Inflammation associated with atherosclerosis, inflammation associated with chronic kidney disease or lung fibrosis.
20

In an embodiment, the fibrotic disorder may be lung fibrosis, liver fibrosis, kidney fibrosis, or fibrosis associated with atherosclerosis.

Additional agents suitable for treating inflammatory disorders, fibrotic disorders and
25 atherosclerosis are well known in the art.

A further aspect of the invention provides a method of identifying an agent that inhibits activity of human DDR1, or a lead compound for identifying an agent that inhibits activity of human DDR1, the method comprising:

30 assaying a test agent for binding to:

- (i) the human DDR1 epitope defined by the amino acid residues Ala279, Gln281, Ala282, Ser335, Pro337, Gly340, Arg341, Val342, Ile365 and Asp367, or
- (ii) the human DDR1 epitope encompassed by the amino acid
35 residues Tyr203 to Tyr209, or
- (ii) the human DDR1 epitope defined by the amino acid residues Met318, Asn321 and Asn325,

wherein a test agent that binds to one of the said DDR1 epitopes may be an agent that inhibits activity of DDR1, or a lead compound for identifying an agent that inhibits activity of DDR1.

- 5 In one embodiment, the assaying step comprises predicting whether the test agent binds to one of the said DDR1 epitopes by molecular modelling *in silico*.

The capability of a test agent or candidate compound to bind to or interact with one of the said DDR1 epitopes may be measured by any method of detecting/measuring a
10 protein/protein interaction or other compound/protein interaction, as discussed further below. Suitable methods include methods such as, for example, yeast two-hybrid interactions, co-purification, ELISA, co-immunoprecipitation and surface plasmon resonance methods. Thus, the candidate compound may be considered capable of binding to the polypeptide or fragment thereof if an interaction may be detected between
15 the candidate compound and the polypeptide or fragment thereof by ELISA, co-immunoprecipitation or surface plasmon resonance methods or by a yeast two-hybrid interaction or copurification method. It is preferred that the interaction can be detected using a surface plasmon resonance method. Surface plasmon resonance methods are well known to those skilled in the art. Techniques are described in, for example,
20 O'Shannessy DJ (1994) "Determination of kinetic rate and equilibrium binding constants for macromolecular interactions: a critique of the surface plasmon resonance literature" *Curr Opin Biotechnol.* 5(1):65-71; Fivash *et al* (1998) "BIAcore for macromolecular interaction." *Curr Opin Biotechnol.* 9(1):97-101; Malmqvist (1999) "BIAcore: an affinity biosensor system for characterization of biomolecular interactions." *Biochem Soc Trans.*
25 27(2):335-40.

The capability of a test agent or candidate compound to bind to or interact with the human DDR1 epitope defined by the amino acid residues Ala279, Gln281, Ala282, Ser335, Pro337, Gly340, Arg341, Val342, Ile365 and Asp367 may also be determined
30 *via* competitive binding assays with antibody 3E3, i.e. an antibody having the heavy and light chain sequences listed in Figure 11.

It is appreciated that screening assays which are capable of high throughput operation are particularly preferred. Examples may include cell based assays and protein-protein
35 binding assays. An SPA-based (Scintillation Proximity Assay; Amersham International) system may be used. For example, an assay for identifying a compound capable of modulating the activity of a protein kinase may be performed as follows. Beads

- comprising scintillant and a substrate polypeptide that may be phosphorylated may be prepared. The beads may be mixed with a sample comprising the protein kinase and ^{32}P -ATP or ^{33}P -ATP and with the test compound. Conveniently this is done in a multi-well (e.g., 96 or 384) format. The plate is then counted using a suitable scintillation counter, using known parameters for ^{32}P or ^{33}P SPA assays. Only ^{32}P or ^{33}P that is in proximity to the scintillant, i.e., only that bound to the polypeptide, is detected. Variants of such an assay, for example in which the polypeptide is immobilised on the scintillant beads *via* binding to an antibody or antibody fragment, may also be used.
- Other methods of detecting polypeptide/polypeptide interactions include ultrafiltration with ion spray mass spectroscopy/HPLC methods or other physical and analytical methods. Fluorescence Energy Resonance Transfer (FRET) methods, for example, well known to those skilled in the art, may be used, in which binding of two fluorescent labelled entities may be measured by measuring the interaction of the fluorescent labels when in close proximity to each other.

In various embodiments, it may be preferred that the test agent is an antibody, an antibody mimic (for example, based upon a non-antibody scaffold), an RNA aptamer, a small molecule, a CovX-body, a natural product, a polypeptide, or a nucleic acid

Preferences for antibodies including antibody fragments, antibody mimics, RNA aptamers, small molecules, a CovX-bodies are described above.

It is appreciated that the identification of a candidate compound that binds to the DDR1 epitope may be an initial step in the drug screening pathway, and the identified compound(s) may be further selected e.g. for the ability to inhibit DDR1 activity. Thus, in an embodiment, the method further comprises the step determining whether the test agent reduces collagen-induced autophosphorylation of DDR1, for example using the methods described herein and known in the art.

It is appreciated that these methods may be a drug screening methods, a term well known to those skilled in the art, and the candidate compound may be a drug-like compound or lead compound for the development of a drug-like compound.

The term "drug-like compound" is well known to those skilled in the art, and may include the meaning of a compound that has characteristics that may make it suitable for use in medicine, for example as the active ingredient in a medicament. Thus, for example, a

drug-like compound may be a molecule that may be synthesised by the techniques of organic chemistry, less preferably by techniques of molecular biology or biochemistry, and is preferably a small molecule, which may be of less than 5000 Daltons and which may be water-soluble. A drug-like compound may additionally exhibit features of selective interaction with a particular protein or proteins and be bioavailable and/or able to penetrate target cellular membranes or the blood:brain barrier, but it will be appreciated that these features are not essential.

The term "lead compound" is similarly well known to those skilled in the art, and may include the meaning that the compound, whilst not itself suitable for use as a drug (for example because it is only weakly potent against its intended target, non-selective in its action, unstable, poorly soluble, difficult to synthesise or has poor bioavailability) may provide a starting-point for the design of other compounds that may have more desirable characteristics.

In an embodiment, the identified compound is modified, and the modified compound is retested. In a related embodiment, an agent having or expected to have similar properties to an agent identified as a result of the method is retested.

It is appreciated that the screening methods can be used to identify agents that may be useful in treating tumours/cancers, or inflammatory disorders, fibrotic disorders, or atherosclerosis. Thus an agent identified as a result of the method may be tested for efficacy in a cell model and/or an animal model of a condition selected from cancer, an inflammatory disorder, a fibrotic disorder, or atherosclerosis.

The cellular model of cancer may be an *in vitro* anti-cancer assay such as a cellular proliferation assay of primary cancer cells or cancer cell lines *in vitro*, as is well known in the art. The non-human animal models of cancer may be, for example, a model of ovarian, lung, breast, colorectal, pancreatic, gastric, oesophageal, renal, biliary, hepatic, cervical, uterine or prostate cancer. The animal model of cancer may be a xenograph model of cancer. Typically, the animal model of cancer is a mouse model of cancer, which may be nude mouse, scid mouse or oncomouse model of cancer. Suitable animal models of cancer are known in the art and include Lewis lung carcinoma subcutaneous implants in mice (homograft in Black 57 mice) or HT29 xenografts subcutaneous implants in nude mice.

A suitable animal model to study fibrosis is bleomycin induced lung fibrosis, as used in a DDR1 study by Avivi-Green *et al* (2006) *Am J Respir Crit Care Med*, 174, 420. A suitable *in vitro* fibrosis model is described by Xu *et al* (2007) *Am J Physiol Renal Physiol*, 293, F631.

5

Suitable atherosclerosis models include LDL-receptor-deficient (*Ldlr*^{-/-}) mouse model, which was also used to study effect of DDR1 on atherosclerosis (e.g., Franco *et al* (2008) *Circ Res*, 102, 1202; Franco *et al* (2009), *Circ Res*, 105: 1141). Other atherosclerosis models can be found in Singh *et al* (2009) *Curr Vasc Pharm*, 7, 75.

10 Suitable *in vitro* models for atherosclerosis include co-culture of arterial wall cells and macrophages, and the *in vitro* model described by Dorweller *et al* (2006) *Thromb Haemost*, 95, 182.

In a further embodiment, an agent identified as a result of the method is further tested for efficacy and safety in a clinical trial of a condition selected from cancer, an inflammatory disorder, a fibrotic disorder, or atherosclerosis. When this part of the method is conducted in the context of a clinical trial, the individuals in the trial may be human patients with cancer or matched controls.

20 The invention may comprise the further step of synthesising and/or purifying the identified compound or the modified compound. The invention may further comprise the step of formulating the compound into a pharmaceutically acceptable composition.

The agent identified, synthesised and/or purified as a result of the method may be packaged and presented for use in treating a condition selected from cancer, an inflammatory disorder, a fibrotic disorder, or atherosclerosis.

Compounds may also be subjected to other tests, for example toxicology or metabolism tests, as is well known to those skilled in the art.

30

Thus the invention includes a method for preparing a compound that may be useful in the treatment of a cancer/tumour an inflammatory disorder, a fibrotic disorder, or atherosclerosis, the method comprising identifying a compound using the screening methods described above and synthesising, purifying and/or formulating the identified compound.

35

The invention also includes a method of making a pharmaceutical composition comprising the step of mixing the compound identified using the methods described above with a pharmaceutically acceptable carrier.

- 5 As used herein, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an antibody" includes a plurality of such antibodies and reference to "the dosage" includes reference to one or more dosages and equivalents thereof known to those skilled in the art, and so forth.
- 10 The listing or discussion in this specification of an apparently prior-published document should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge. Any document referred to herein is hereby incorporated by reference.
- 15 Preferred, non-limiting examples which embody certain aspects of the invention will now be described, with reference to the following figures:

Figure 1. The anti-DDR1 mAbs block collagen-induced DDR1 phosphorylation.

DDR1b was transiently expressed in HEK293 cells and the cells were stimulated for 90 min at 37° C with 10 µg/ml collagen I in the presence or absence of the indicated anti-DDR1 mAbs (at 10 µg/ml). Aliquots of cell lysates were analysed by SDS-PAGE and Western blotting. The blots were probed with anti-phosphotyrosine (anti-PY) mAb 4G10 (upper blot) and re-probed with anti-DDR1 Abs (lower blot). Control, mouse IgG1 isotype control Ab. The experiment was performed three times with very similar results.

25

Figure 2. Selected Fab fragments block collagen-induced DDR1 phosphorylation.

DDR1b was transiently expressed in HEK293 cells. The cells were stimulated for 90 min at 37° C with 10 µg/ml collagen I in the presence or absence of the indicated anti-DDR1 mAbs or Fab fragments (at 10 µg/ml). Aliquots of cell lysates were analysed by SDS-PAGE and Western blotting. The blots were probed with anti-phosphotyrosine (anti-PY) mAb 4G10 (upper blot) and re-probed with anti-DDR1 Abs (lower blot). The experiment was performed three times with very similar results.

30

Figure 3. The anti-DDR1 mAbs bind to the DS-like domain and do not inhibit ligand

35 **binding. (A)** ELISA showing binding of the indicated anti-DDR1 mAbs to recombinant DDR proteins immobilised onto 96-well plates. Shown is a representative of three independent experiments, each carried out in duplicates. **(B)** Solid-phase binding assay

with recombinant DDR1-Fc protein added for 3 h at room temperature to 96-well plates coated with either κ -casein or collagen peptide III-23 (Xu et al., 2011). DDR1-Fc was preincubated with the indicated anti-DDR1 mAbs before addition to the wells. Bound DDR1-Fc was detected with anti-human Fc and measured as absorbance at 492 nm.

- 5 Shown is a representative of three independent experiments, each carried out in triplicates.

Figure 4. (A) Overall structure of the DDR1-3E3 Fab complex. The 3E3 Fab fragment is shown as a surface (tan, light chain; grey, heavy chain) and DDR1 is shown as a cartoon (cyan, DS domain; green, DS-like domain; red, collagen-binding loops (Carafoli et al., 2009); orange, disulphide bridges). A calcium ion is shown as a magenta sphere and the two *N*-linked glycans are shown as light blue sticks. The *N*- and *C*-termini of the DDR1 construct are indicated. The β -strands of the jelly roll in the DS and DS-like domains are numbered 1-8 and the extra β -strands in the DS-like domain are labelled a-e. (B) Superposition of the DS domain (cyan) and the DS-like domain (green) of DDR1. (C) Detailed structure of the interface between the DS domain (cyan) and the DS-like domain (green) in DDR1. Selected residues are shown in atomic detail and labelled. Hydrogen bonds are indicated by dashed lines.

20 **Figure 5.** Alignment of selected DDR1 and DDR2 sequences (*Homo sapiens* DDR1, Q08345; *Mus musculus* DDR1, Q03146; *Danio rerio* DDR1, XP_001345829; *Xenopus tropicalis* DDR1, XP_002939505; *Homo sapiens* DDR2, Q16832; *Mus musculus* DDR2, Q62371; *Danio rerio* DDR2, XP_684261; *Xenopus tropicalis* DDR2, XP_002933824). The numbers above the alignment refer to the human DDR1 sequence. Conserved residues are in bold and cysteines are in orange. Predicted *N*-linked glycosylation sites are underlined. The secondary structure elements of the DDR1 structure are indicated above the alignment. Red inverted triangles indicate key collagen-binding residues in DDR2 (Carafoli et al., 2009). Magenta filled circles indicate calcium ligands in DDR1. Pink diamonds indicate residues contributing to the conserved surface patch in the DS domain. The seven linear and non-conservative human-to-mouse substitutions in the DS-like domain are boxed in blue and labelled epi1-7.

Figure 6. Superposition of DDR1 (cyan, DS domain; green DS-like domain) and the DS domain pair of neuropilin-1 (Nrp1, magenta) (Vander Kooi et al., 2007). The DDR1 DS domain was fitted to the first DS domain (b1) of Nrp1. The DDR1 DS-like domain and the second DS domain (b2) of Nrp1 are related by a $\sim 60^\circ$ rotation about a vertical axis.

Figure 7. (A) The lattice contact resulting in a 2-fold symmetric DDR1 dimer (see text). The DDR1 molecule on the left is in cyan (DS domain) and green (DS-like domain); the DDR1 molecule on the right is in grey with the collagen-binding loops (Carafoli et al., 2009) in red. The 2-fold symmetry axis is vertical. Selected residues are shown in atomic detail (pink, conserved surface patch in the DS domain). (B) Wild-type DDR1b or the indicated mutants were transiently expressed in HEK293 cells. The cells were stimulated for 90 min at 37° C with collagen I at the indicated concentrations (in µg/ml). Aliquots of cell lysates were analysed by SDS-PAGE and Western blotting. The blots were probed with anti-phosphotyrosine (anti-PY) mAb 4G10 (upper blot) and re-probed with anti-DDR1 Abs (lower blot). The experiment was performed three times with very similar results.

Figure 8. Detailed structure of the DDR1-3E3 Fab interface. The 3E3 Fab fragment is shown as a semitransparent surface (tan, light chain; grey, heavy chain) and the DDR1 region interacting with the Fab is shown as a green cartoon. Selected interface residues are shown in atomic detail and labelled. Hydrogen bonds are indicated by dashed lines.

Figure 9. (A) Epitope mapping of anti-DDR1 mAbs. (A) Wild-type DDR1b or the indicated mutants were transiently expressed in HEK293 cells. The cells were stained on ice with 10 µg/ml of the indicated anti-DDR1 mAbs or mouse IgG1 isotype control Ab followed by FITC-conjugated goat-anti mouse IgG and analysis by flow cytometry. Binding of isotype control Ab is shown by the filled grey histograms. Shown are representative data of at least three experiments for each DDR1 mutant. (B) Surface representation of DDR1 structure showing the location of mAb epitopes (epi1: 3G10, 3H10, 7A9; epi5: 3E3; epi 6: 1F7, 1F10). The DS and DS-like domains are in cyan and green, respectively. The collagen binding site is in red. The conserved surface patch (Arg32, Leu99, Leu152, Tyr183) is in pink.

Figure 10. Mean fluorescence values of the flow cytometry data shown in Figure 9. DDR1b wild-type or the indicated DDR1 mutants were transiently expressed in HEK293 cells. The cells were stained on ice with 10 µg/ml of the indicated anti-DDR1 mAbs or mouse IgG1 isotype control Ab followed by FITC-conjugated goat-anti mouse IgG and analysis by flow cytometry.

Figure 11. Anti DDR1 mAb 3E3 sequence: V regions amplified as in Orlandi *et al*, (1989). *PNAS USA* **86**:3833-7. Residues encoded by the PCR primers are underlined. Constant regions are from PDB entry 12E8 (mouse IgG1) with one change (W)

Figure 12. mAb 1F10 blocks collagen-induced DDR1 phosphorylation in cancer

cell lines. The cell lines are DLD-1 (colon carcinoma cell line) and the breast cancer cell lines SKBr-3, MCF-7 and T47D. Cells were stimulated, or not, with 10 µg/ml collagen (rat tail collagen I, Coll I,) for 90 minutes at 37°C in the presence or absence of 10 µg/ml anti-DDR1 mAb 1F10, as indicated. DDR1 was immunoprecipitated with rabbit anti-DDR1 antibodies and the immunoprecipitates were analysed by SDS-PAGE and Western blotting. The blots were probed with anti-phosphotyrosine (anti-PY) mAb 4G10 (upper blots) and reprobed with anti-DDR1 Abs (lower blots).

Example 1: Structure of the extracellular region of discoidin domain receptor 1 bound to an inhibitory Fab fragment reveals features important for signalling

Introduction

Receptor tyrosine kinases (RTKs) control many fundamental cellular processes, such as cell proliferation, differentiation, migration and metabolism (Lemmon and Schlessinger, 2010). RTK activity is normally tightly controlled and dysregulation of RTK activity is associated with many human cancers and other pathologies. Ligand binding to the extracellular region of RTKs leads to autophosphorylation of their cytoplasmic kinase domains, creating docking sites for effectors of downstream signalling. The two major strategies for controlling unwanted RTK activity in human patients are inhibition by monoclonal antibodies (mAbs) directed against their extracellular regions or by small molecules targeting the kinase active site (Adams and Weiner, 2005; Gschwind et al., 2004).

The discoidin domain receptors, DDR1 and DDR2, are RTKs that are activated by several types of triple-helical collagen, a major component of the animal extracellular matrix (Leitinger, 2011; Shrivastava et al., 1997; Vogel et al., 1997). The DDRs are widely expressed in mammalian tissues and have important roles in embryo development and human disease (Vogel et al., 2006). For example, DDR1 is essential for mammary gland development (Vogel et al., 2001) and DDR2 for the growth of long bones (Labrador et al., 2001). DDR2 mutations in humans cause a rare, severe form of dwarfism (Ali et al., 2010; Bargal et al., 2009). The DDRs are also implicated in cancer, fibrotic diseases, atherosclerosis and arthritis (Vogel et al., 2006). Mechanistically, the DDRs have several features that distinguish them from other RTKs. Compared with the rapid response of typical RTKs to their soluble ligands (e.g. growth factors), collagen-induced DDR autophosphorylation is slow and sustained (Shrivastava et al., 1997; Vogel

et al., 1997). Furthermore, Src kinase plays an essential role in DDR activation (Ikeda et al., 2002).

Both DDRs are composed of an N-terminal discoidin (DS) domain (Baumgartner et al., 1998), followed by a unique sequence of just over 200 amino acids, a transmembrane domain, a large cytosolic juxtamembrane domain and a C-terminal tyrosine kinase domain. Collagen binds to the DS domain and the structural determinants of the DDR-collagen interaction have been extensively studied (Carafoli et al., 2009; Ichikawa et al., 2007; Konitsiotis et al., 2008; Leitinger, 2003; Xu et al., 2011). The remainder of the extracellular region has not been characterised structurally. Our unpublished structure prediction, by threading analysis (Lobley et al., 2009), suggests that it may contain a domain belonging to the same superfamily as the DS domain, which we have termed the DS-like domain (and this is confirmed below).

How collagen binding results in DDR activation is a major unresolved question. DDR1 can be activated by short collagen-like peptides, showing that DDR clustering by multivalent collagen assemblies (e.g. microfibrils) is not essential for activation (Konitsiotis et al., 2008). The DDRs are constitutive dimers at the cell surface and residues within the transmembrane helix are required for signalling (Noordeen et al., 2006). In fact, a comprehensive analysis has shown that the DDRs have the highest propensity of transmembrane helix self-interactions in the entire RTK superfamily (Finger et al., 2009). Therefore, the conformational changes resulting from collagen binding are likely to occur in the context of a stable DDR dimer. Our crystal structure of a DDR2 DS-collagen peptide complex (Carafoli et al., 2009) revealed a 1:1 complex and did not clarify how collagen binding affects the conformation of the DDR dimer. Here, we report the functional characterisation of a set of novel inhibitory anti-DDR1 mAbs and the crystallisation of the almost complete extracellular region of DDR1 bound to a mAb Fab fragment. The crystal structure led to the discovery of DDR1 residues that are required for signalling even though they are not part of the known collagen binding site. These results provide new insight into the process of DDR1 activation.

Experimental procedures

DNA Constructs and Site-Directed Mutagenesis

DNAs coding for full-length DDR1 mutants (R32E, L152E, L247E/R248E, human to mouse substitutions for epitope mapping) were generated by strand overlap extension PCR using a cDNA of human DDR1 as a template (Leitinger, 2003). The PCR primers used to generate these constructs are available on request. The amplified DNAs were

cloned into the mammalian expression vectors pcDNA3.1/Zeo (Invitrogen) or pRK5 (BD Pharmingen). DNA coding for the DDR1 construct used for crystallography was generated by PCR and cloned into a modified pCEP-Pu vector adding a C-terminal His-tag (Kohfeldt et al., 1997). All PCR-derived DNA constructs were verified by sequencing.

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Production of Soluble DDR1 Proteins

The following proteins were produced as described (Leitinger, 2003): His-DDR1 contains the entire extracellular region of human DDR1 (residues 19-416 of UniProt entry Q08345). His-DDR2 contains the entire extracellular region of human DDR2 (residues 10 22-398 of UniProt entry Q16832). His-DS-DDR1 and His-ΔDS-DDR1 are deletion constructs based on His-DDR1: His-DS-DDR1 lacks the DS-like domain (Δ201-369) and His-ΔDS-DDR1 lacks the DS domain (Δ31-185). DDR1-Fc contains the entire DDR1 extracellular region fused to a C-terminal human IgG1 Fc sequence (Leitinger, 2003; Xu et al., 2011).

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The DDR1 construct for crystallography contains the DS and DS-like domains of human DDR1 (residues 30-367) fused to a C-terminal His-tag (AAAHHHHHH). A vector-derived APLA sequence is present at the N-terminus of the mature protein. The protein was produced in human embryonic kidney HEK293 c18 cells (ATCC). The cells were grown at 37° C with 5% CO₂ in Dulbecco's modified Eagle's medium/F12 (Invitrogen) containing 10% fetal bovine serum, 2 mM glutamine, 10 U/ml penicillin, 100 µg/ml streptomycin and 250 µg/ml geneticin. The cells were transfected with the pCEP-Pu expression plasmid using Fugene (Roche Diagnostics) and selected with 1 µg/ml puromycin (Sigma). Confluent cells in a HYPERFlask (Corning) were washed twice with PBS and incubated 25 with serum-free medium for 3-4 weeks, with weekly medium exchanges. The pooled serum-free conditioned medium was loaded onto a 5-ml HisTrap column (GE Healthcare) using an Äkta Purifier (GE Healthcare). The protein was eluted with 500 mM imidazole in PBS, concentrated using a Vivaspin centrifugal device (Sartorius), and further purified on a Superdex 200 HR10/30 size exclusion chromatography column (GE 30 Healthcare) with Tris-buffered saline (TBS) (25 mM Tris, 150 mM NaCl, 2 mM KCl, pH 7.4) as the running buffer. The final yield from 1 litre of cell culture medium was 15 mg of DDR1 protein.

Generation of anti-DDR1 Antibodies

35 To prepare an untagged antigen for immunisation, 500 µg of His-DDR1 (Leitinger, 2003) were digested with 25 U EKMax enterokinase (Invitrogen) for 16 h at 4° C. EKMax was removed with EK-Away resin (Invitrogen) according to the manufacturer's protocol.

Uncleaved His-DDR1 and the cleaved tag were removed with TALON metal affinity beads (Clontech). The untagged DDR1 protein was dialysed against PBS and concentrated to 2 mg/ml by ultrafiltration. Mouse anti-DDR1 monoclonal antibodies (mAbs) were generated by immunising female BALB/c mice with the untagged DDR1 protein. Three days after the final boost one mouse was sacrificed to obtain splenocytes for hybridoma production by standard procedures. Hybridoma cell supernatants were screened against DDR1-Fc and His-DDR1 proteins by enzyme-linked immunosorbent assay (ELISA). Reactive hybridoma supernatants were further screened for recognition of native DDR1 by cell-based ELISA, using HEK293 cells expressing full-length DDR1. Positive hybridoma cells were subcloned by limited dilution and screened as above. The isotype of each mAb was determined by standard methods. All mAbs are of the IgG1 isotype, with the exception of mAb 1F10, which is IgG2b.

Antibody and Fab Fragment Production

Hybridoma cells were grown at 37° C with 5% CO₂ in RPMI-1640 medium (Invitrogen) containing 10% fetal bovine serum, 1 mM sodium pyruvate, 10 U/ml penicillin, 100 µg/ml streptomycin and 1 µg/ml fungizone (Invitrogen). The serum concentration was gradually reduced to 5% in a final culture volume of 1 litre. The hybridoma cell culture supernatant was loaded onto a 2 x 1-ml HiTrap rProtein A column (GE Healthcare). The mAbs were eluted with Immunopure gentle elution buffer (Pierce) and dialysed against TBS. Fab fragments were generated with a Fab Preparation Kit (Pierce) according to the manufacturer's protocol. Briefly, 8 mg of mAb were incubated overnight at 37° C with activated papain immobilised on agarose resin. The Fc fragment and undigested mAb were removed using a 1-ml HiTrap rProtein A column (GE Healthcare), yielding ~3 mg of Fab fragment. The Fab fragments used in co-crystallisation experiments were further purified by size exclusion chromatography on a Superdex 200 HR10/30 column (GE Healthcare) with TBS as the running buffer.

mAb cDNA Synthesis and Sequencing

Total RNA was prepared from ~10⁷ 3E3 hybridoma cells using the RNeasy Mini Kit (Qiagen). The RNA was reverse-transcribed and cDNA fragments encoding the heavy- and light-chain variable regions of the mAb were amplified using the SuperScript III One-Step RT-PCR system (Invitrogen) and suitable universal primers (Orlandi et al., 1989). The PCR products were gel-purified and sequenced using the same primers. The mAb residues are numbered according to (Al-Lazikani et al., 1997).

DDR1 Activation Assay

The assay was performed as described (Leitinger, 2003). Briefly, HEK293 cells were grown in 12-well tissue culture plates and transfected with 2 µg/well of DDR1 wild-type or mutant plasmid DNA using calcium phosphate precipitation. 24 h after transfection the

5 cells were incubated with serum-free medium for 16 h. Cells were then stimulated with 10-50 µg/ml acid-soluble rat tail collagen I (Sigma) for 90 min at 37° C before being lysed. In the inhibition experiments, anti-DDR1 mAbs or their Fab fragments were added together with collagen I, without prior incubation. Aliquots of the cell lysates were subjected to SDS-PAGE and blotted onto nitrocellulose membranes. The blots were first

10 probed with an mouse anti-phosphotyrosine mAb (clone 4G10, Upstate Biotechnology) followed by a horseradish peroxidase-conjugated sheep anti-mouse Ig (Amersham Biosciences). Detection was done by Enhanced Chemiluminescence Plus (Amersham Biosciences) using an Ettan DIGE Imager (GE Healthcare). To reprobe the blots, the membranes were treated with Ab stripping solution (Alpha Diagnostic International),

15 followed by incubation with rabbit anti-DDR1 Ab (SC-532, Santa Cruz Biotechnology) and finally goat horseradish peroxidase-conjugated anti-rabbit Ig (P0448, DAKO).

ELISA and Solid Phase Binding Assays

Recombinant DDR proteins, diluted to 10 µg/ml in 50 mM Tris, 100 mM NaCl, pH 8.5,

20 were coated in 50 µl aliquots onto Maxisorp 96-well plates (Nalgene NUNC) overnight at room temperature. The wells were blocked with 150 µl of incubation buffer (PBS containing 40 µg/ml bovine milk κ -casein (Sigma) and 0.05% Tween-20) for 1 h at room temperature. Anti-DDR1 mAbs were added at 30 µg/ml in 50 µl aliquots and incubated for 1.5 h at room temperature. The wells were washed 6 times with incubation buffer,

25 followed by the addition of horseradish peroxidase-conjugated sheep anti-mouse Ig (Amersham Biosciences, 1:1000 dilution in incubation buffer) for 1.5 h at room temperature. After 6 washes as above, bound mAbs were detected with 75 µl/well of 500 µg/ml o-phenylenediamine dihydrochloride (Sigma-Aldrich) in 50 mM citrate-phosphate pH 5.0. The reaction was stopped after 3-5 minutes with 50 µl/well of 3 M H₂SO₄. The

30 absorbance at 492 nm was measured using a Sunrise 96-well plate reader (Tecan).

To measure DDR1 binding to the collagen-derived peptide III-23, Immulon 2 HB 96-well plates (Fisher Scientific) were coated overnight at room temperature with 10 µg/ml III-23 in 10 mM acetic acid (Xu et al., 2011). The wells were then blocked in incubation buffer

35 as described above. DDR1-Fc protein was incubated with anti-DDR1 mAbs for 30 minutes at room temperature before being added to the wells. After 3 h of incubation at room temperature and 6 washes with incubation buffer, bound DDR1-Fc was detected

with horseradish peroxidase-conjugated goat anti-human Fc (Jackson ImmunoResearch Laboratories, 1:3333 dilution), added for 1 h at room temperature. The assay was completed as described above.

5 Flow Cytometry

HEK293 cells were grown in 6-well plates and transfected with 5 µg/well of DDR1 wild-type or mutant plasmid DNA using calcium phosphate precipitation. 48 h after transfection, the cells were dissociated with non-enzymatic cell dissociation solution (Sigma) and resuspended in PBS containing 1% BSA. The cells were incubated with
10 primary mAb or mouse IgG1 isotype control Ab (Cambridge Bioscience) at 10 µg/ml in 100 µl PBS/BSA for 30 minutes on ice, followed by three washes with PBS/BSA and incubation with FITC-conjugated goat anti-mouse IgG (F-9006, Sigma) for 30 min on ice. After three washes as above, the cells were resuspended in 2% formaldehyde in PBS and analysed on a FACS Calibur flow cytometer using Cell Quest Pro software (Becton
15 Dickinson Biosciences).

Crystal Structure Determination

The purified DDR1 protein for crystallography and the 3E3 Fab fragment were mixed in an equimolar ratio and incubated on ice for 30 minutes. The solution was subjected to
20 size exclusion chromatography on a Superdex 200 HR10/30 column (GE Healthcare) with TBS as the running buffer. The DDR1-3E3 Fab complex eluted as a single peak and was concentrated to 6 mg/ml. Sitting drop vapour diffusion crystallisation screens were set up using a Mosquito nanolitre robot (TTP LabTech). Crystals were obtained after 1-2 days at room temperature using 2% Tacsimate pH 5.0 (Hampton Research), 100 mM
25 sodium citrate tribasic dihydrate pH 5.6, 20% PEG 3350 as precipitant. Crystals were flash-frozen in liquid nitrogen after a brief soak in mother liquor supplemented with 25% glycerol. Diffraction data were collected at 100 K on station I02 at the Diamond Light Source (Oxfordshire, UK). The data were processed with MOSFLM (www.mrc-lmb.cam.ac.uk/harry/mosflm) and programs of the CCP4 suite (CCP4, 1994). The
30 DDR1-3E3 Fab structure was solved by molecular replacement with PHASER (McCoy et al., 2005; Storoni et al., 2004) using as search models the DDR2 DS domain (PDB entry 2wuh) and a Fab fragment of an Ab directed against neuropilin-2 (PDB entry 2qqk). The electron density map calculated from the correctly positioned search models showed weak density for several β-strands in the DS-like domain, which were used to place the
35 related F5/8 type C domain of galactose oxidase (PDB entry 1k3i) as an aid for model building. The model was built with O (Jones et al., 1991) and refined with CNS (Brunger

et al., 1998). Crystallographic statistics are summarised in **Table 1**. The figures were made with PyMOL (www.pymol.org).

Results

5 Generation and Characterisation of anti-DDR1 mAbs

We immunised mice with a recombinant protein spanning the entire extracellular region of human DDR1 and obtained seven anti-DDR1 mAbs. All seven mAbs were found to inhibit the collagen-induced autophosphorylation of DDR1 expressed in HEK293 cells (**Figure 1**) and this inhibitory activity was retained by Fab fragments generated from five
10 of the seven mAbs (**Figure 2**). In an ELISA assay with soluble protein constructs of the DDR1 extracellular region, all seven mAbs bound to the membrane-proximal DS-like domain (His- Δ DS-DDR1), but not to the membrane-distal DS domain containing the collagen binding site (His-DS-DDR1) (**Figure 3A**). This result suggested that the inhibitory activity of the mAbs on cells was unlikely to be the result of a block of collagen
15 binding. Indeed, when we tested a subset of mAbs in a direct collagen binding assay, we found that binding of DDR1-Fc to a high-affinity collagen peptide (III-23, (Xu et al., 2011)) was not affected by the addition of mAbs (**Figure 3B**). The combined results demonstrate that the novel anti-DDR1 mAbs inhibit DDR1 function by interfering with the signal transduction process resulting from collagen binding.

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Crystal Structure of a DDR1-Fab Complex

For several years, we had attempted unsuccessfully to obtain diffracting crystals of the extracellular regions of DDR1 or DDR2. Fab fragments of mAbs have been instrumental in facilitating the crystallisation of many recalcitrant proteins (Nettlehip et al., 2008). We
25 therefore screened the Fab fragments of six anti-DDR1 mAbs for complex formation and co-crystallisation with the extracellular region of DDR1 (the 1F7 Fab could not be used because it aggregated in solution). Because the His-DDR1 construct used for mAb generation included the juxtamembrane region that is predicted to be unstructured, we produced a new construct terminating at the predicted C-terminus of the DS-like domain,
30 Asp367. All Fab fragments bound to this shortened DDR1 construct, as determined by analytical size exclusion chromatography (data not shown). We obtained crystals of the DDR1-3E3 Fab complex and determined its structure at a resolution of 2.8 Å (**Table 1**).

Table 1. Crystallographic Statistics of the DDR1-3E3 Fab Structure

Data collection	
Space group	C222 ₁
Unit cell dimensions	
a, b, c (Å)	102.51, 251.48, 75.37
α, β, γ (°)	90, 90, 90
Asymmetric unit content	1:1 DDR1-3E3 Fab complex
Solvent content (%)	57
Resolution (Å)	50-2.8 (2.95-2.80) ^a
R _{merge}	0.079 (0.429)
<I/σ(I)>	14.3 (3.8)
Completeness (%)	98.7 (98.9)
Multiplicity	6.0 (6.1)
Refinement	
Resolution (Å)	20-2.8
Reflections	24035
Protein atoms	2700 (DDR1) + 3169 (3E3 Fab)
Solvent atoms	2 Ca ²⁺ + 14 H ₂ O
R _{work} /R _{free}	0.216/0.286
R.m.s. deviation bonds (Å)	0.007
R.m.s. deviation angles (°)	1.4
Average B-factor (Å ²)	53.6
Ramachandran plot (%) ^b	91.3/98.1

^a Values in parantheses are for the highest resolution shell.

^b Percentage of residues in favoured and allowed regions (Chen et al., 2010).

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The extracellular region of DDR1 revealed by the crystal structure is a compact structure measuring approximately 70 Å x 50 Å x 40 Å (**Figure 4A, Figure 5**). The DS and DS-like domains are arranged such that the long axes of their β-barrels are roughly perpendicular to each other, with a substantial (703 Å²) interface between them. This arrangement is reminiscent of the tandem DS domains in neuropilins (Appleton et al., 2007; Vander Kooi et al., 2007), but the second domain is rotated differently in the two proteins (**Figure 6**). The N- and C-termini of the crystallised DDR1 construct are located on the same face of the molecule near the interdomain linker. In the intact receptor, the C-terminus of the crystallised construct would be linked to the transmembrane helix by the 50-residue juxtamembrane region. The DDR1 DS domain is very similar (r.m.s.

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deviation of 0.61 Å for 156 Cα atoms) to the DDR2 DS domain, which was previously crystallised in complex with a collagen-like peptide (Carafoli et al., 2009). The collagen binding loops of the DDR1 DS domain, which are opposite the DS-like domain, have weak electron density and high temperature factors, suggesting that they are quite mobile in the absence of the collagen ligand. The 3E3 Fab fragment is bound near the C-terminus of the DS-like domain, distant from the collagen binding site (for a description of the epitope, see below).

Structure of the DS-like Domain

As predicted from threading analysis, the DS-like domain of DDR1 belongs to the F5/8 type C superfamily. A search with the program SSM (Krissinel and Henrick, 2004) showed that the DS-like domain is most closely related to family 32 carbohydrate binding modules (CBMs) (Boraston et al., 2004), but a pairwise alignment of the DS and DS-like domains of DDR1 gave only a marginally lower Z-score and a r.m.s. deviation of 3.0 Å for 120 aligned Cα atoms (**Figure 4B**). To facilitate the comparison of the DS and DS-like domains, the eight β-strands that are common to both domains have been labelled β1-β8. The DS-like domain contains five additional strands, labelled βa-βe, in a long insertion between β1 and β2. Both domains are characterised by two antiparallel β-sheets with jellyroll topology (β1-β2-β7-β4 sheet and β5-β6-β3-β8 sheet). At one end of the β-barrel (the “bottom”) the β2-β3 and β6-β7 loops cross over between the sheets and create a relatively flat surface. At the other end (the “top”), several long and irregular loops protrude from the barrel. In the DS domain, these loops constitute the collagen binding site (Carafoli et al., 2009; Ichikawa et al., 2007; Leitinger, 2003). In the DS-like domain, they contribute the extra strands βa-βe, two N-linked glycosylation sites (Asn211 and Asn260) and a calcium binding site. The calcium ion is coordinated by the side chains of Asp233 and Glu361, as well as by three main chain carbonyl groups; an analogous calcium coordination is seen in many family 32 CBMs (Boraston et al., 2004). It is noteworthy that the glycosylation site at Asn211 and the calcium ligands are strictly conserved in all vertebrate DDRs (**Figure 5**). A second ion in the DS-like domain was also modelled as calcium, but this ion appears to be bound more weakly and may be a crystal artefact (not shown). The DS-like domain of DDR1 contains three cysteines: Cys303 and Cys343, which form a deeply buried disulphide bridge linking the adjacent β4 and β7 strands, and Cys287, which is unpaired and also buried. A previous study suggested that Cys303 and Cys343 may be involved in the covalent dimerisation of DDR1 (Abdulhussein et al., 2008). However, the DS-like domain would have to unfold for these two residues to become available for intermolecular disulphide bridges. The

disulphide-linked dimers seen in that study therefore are more likely to have resulted from oxidation following denaturation.

The interface between the DS and DS-like domains of DDR1 is formed between the
 5 bottom of the DS domain, in particular the β 4- β 5 and β 6- β 7 loops, and the long convoluted insertion between strands β 1 and β 2 of the DS-like domain (**Figure 4C**). A key interface residue is Trp187, which is located in the short linker between the two domains and which interacts with residues of both the DS domain (Leu94 and Val160) and the DS-like domain (Leu191, Leu192, Leu228, Ala232). With the exception of
 10 Leu192 and Ala232, these residues are strictly conserved in all vertebrate DDRs (**Figure 5**). Also conserved is an ion pair spanning the interface, involving Arg124 of the DS domain and Asp216 of the DS-like domain. Additional interdomain contacts are made between the 134-138 and 245-253 loops (**Figure 4C**). The size of the interface ($703 \text{ \AA}^2$) and the conservation of key interface residues suggest that the domain arrangement
 15 seen in the present structure is stable and representative of DDRs in general.

DS Domain Residues Required for DDR1 Signalling

The DDRs are believed to be constitutive dimers at the cell surface (Mihai et al., 2009; Noordeen et al., 2006). The soluble extracellular region of DDR1 is monomeric
 20 (Leitinger, 2003), but because the high protein concentration in the crystallisation buffer might favour a weak dimer association, we inspected the crystal lattice for DDR1 dimers. There was only one plausible dimer. The interface between the two DDR1 molecules in this dimer, which has a total area of $791 \text{ \AA}^2$, is dominated by two identical, symmetry-related, contacts. In each of these contacts, four strictly conserved DS domain residues
 25 (Arg32, Leu99, Leu152, Tyr183) interact with Leu247 and Arg248 of the other DDR1 molecule (**Figure 7A**). To test whether these DDR1 regions are required for function, we expressed three DDR1 mutants (R32E, L152E and L247E/R248E) in HEK293 cells and measured their collagen-induced autophosphorylation. The R32E and L152E mutations abrogated DDR1 autophosphorylation, whereas the L247E/R248E double mutation had
 30 no effect (**Figure 7B**). Very similar results were obtained with DDR2 (data not shown). The normal activity of the L247E/R248E mutant indicates that the DDR1 crystal lattice "dimer" does not correspond to the signalling state of the receptor. The loss of activity in the R32E and L152E mutants demonstrates that this conserved surface region in the DS domain, which is distant from the high-affinity collagen binding site, is required for DDR1
 35 signalling.

Epitopes of Anti-DDR1 mAbs

The 3E3 epitope is formed from three regions of the DDR1 DS-like domain, which are discontinuous in sequence, but contiguous in space: the start of $\beta 3$ (Ala279, Gln281, Ala282), the $\beta 6$ - $\beta 7$ loop (Ser 335, Pro337, Gly340, Arg341, Val342) and the very end of the DS-like domain (Ile365, Asp367) (**Figure 8**). The 3E3 Fab uses predominantly aromatic residues to recognise this epitope: Thr30, Phe32, Tyr34, Tyr49 and Leu50 of the light chain; and Ile31, Trp33, Tyr52, Tyr56 and Tyr96 of the heavy chain. In total, the DDR1-3E3 interface measures 696 Å², of which ~60% are accounted for by contacts involving the heavy chain. Without wishing to be bound by any theory, the finding that mAb 3E3 binds close to the C-terminus of the DS-like domain suggests that it may inhibit DDR1 function by preventing the association of the DS-like domains and/or juxtamembrane regions in the signalling DDR1 dimer.

To better understand how the anti-DDR1 mAbs inhibit DDR1 function, it was of interest to determine the epitopes of the other mAbs as well. We therefore made a series of DDR1 mutants, which targeted all linear and non-conservative human-to-mouse substitutions in the DS-like domain (**Figure 5**). These DDR1 mutants were expressed in HEK293 cells and mAb binding was measured by flow cytometry (**Figure 9A**, **Figure 10**). The epi7 mutant (R341H/A343G) was consistently expressed at lower levels than the wild-type protein, suggesting that the mutation may have compromised the DDR1 structure. All other mutants were expressed at similar levels to the wild-type protein. Saturating concentrations of all but one mAbs gave similar fluorescence profiles for wild-type DDR1. The single exception was 1F10, which is a different isotype (IgG2b) than the other anti-DDR1 mAbs (IgG1) and therefore may be detected less well by the secondary Ab.

Four of the DDR1 mutants (epi2, epi3, epi4, epi7) showed unperturbed binding of all seven mAbs. The epi1 mutation (203-YLSEAVY to QLSEVMVH) abolished binding of mAbs 3G10, 3H10 and 7A9. The epi5 mutation (A279T/A282T) reduced binding of mAb 3E3, consistent with the crystal structure, which shows that Ala279 and Ala282 make close contacts with the 3E3 Fab fragment (**Figure 8**). The epi6 mutation (M318V/N321A/N325S) abolished binding of mAbs 1F7 and 1F10. Binding of mAb 5D5 was not affected by any of the mutations and we assume that the 5D5 epitope is a combination of the linear sequence motifs targeted in our experiments. In summary, the six inhibitory anti-DDR1 mAbs for which the epitopes could be defined bind to three different regions of the DS-like domain that are all >50 Å away from the collagen binding site in the DS domain (**Figure 9B**).

Discussion

In this Example, we report three major findings that advance the mechanistic understanding of DDR signalling: First, crystal structure analysis has revealed that the extracellular region of DDRs consists of two structurally related domains, a collagen-binding DS domain and a DS-like domain. Second, we have generated anti-DDR1 mAbs that inhibit collagen-induced DDR1 activation by binding to the DS-like domain. Third, we have identified a conserved surface patch in the DS domain that is distinct from the collagen binding site, yet required for DDR activation. These results are integrated into a working model of how collagen binding might alter the extracellular structure of DDRs and thereby lead to receptor activation.

The N-terminal domain of DDRs has long been recognised as a member of the DS superfamily (e.g., Johnson et al., 1993; Karn et al., 1993) and its role in collagen binding is understood in atomic detail (Carafoli et al., 2009; Ichikawa et al., 2007). Our new crystal structure shows that the second DDR domain is a distant relative of the DS domain, termed the DS-like domain. Tandem repeats of DS domains occur in a number of secreted and cell surface proteins (Baumgartner et al., 1998; Kiedziarska et al., 2007). In the blood coagulation factors V and VIII, the two DS domains are arranged side-by-side with limited contacts between them, so that their top loops can both interact with the same cell membrane (Adams et al., 2004; Ngo et al., 2008; Shen et al., 2008). In neuropilin-1 and -2, the two DS domains are related by a $\sim 90^\circ$ rotation and form a compact structure, as in DDR1 (Appleton et al., 2007; Vander Kooi et al., 2007). This angled arrangement in DDR1 results in the C-terminus of the DS-like domain emerging near the interdomain linker. The presumably unstructured juxtamembrane region of DDR1 linking the DS-like domain to the transmembrane helix (residues 368-417) contains 12 prolines and a number of predicted *N*- and *O*-linked glycosylation sites. If fully extended, it would project the DS and DS-like domains of DDR1 ~ 150 Å from the cell surface. The juxtamembrane regions of other DDRs are similarly long, ranging from 32 to 74 residues.

mAbs directed against RTKs are invaluable tools for research and have been developed into successful therapeutics (Adams and Weiner, 2005). We have characterised seven anti-DDR1 mAbs that inhibit DDR1 function by binding to various epitopes in the DS-like domain. Notably, Fab fragments derived from these mAbs were equally effective as DDR1 inhibitors. No mAbs were obtained that bind to the DS domain, possibly reflecting the higher degree of surface conservation in that domain (not shown). In agreement with

their epitope locations, the mAbs inhibit DDR1 function without blocking collagen binding. We think that they do so by preventing the proximity of the two DS-like domains and the following juxtamembrane regions in the collagen-bound, signalling, state of the DDR1 dimer (Noordeen et al., 2006). Deletion of the DS-like domain or juxtamembrane region of DDR1 results in receptors that are not trafficked to the cell membrane, so the contribution of these regions to signalling could not be studied (Noordeen et al., 2006). Remarkably, however, the DS-like domain of DDR2 could be deleted without abrogating collagen-induced receptor autophosphorylation (Leitinger, 2003), suggesting that the DS-like domain is not making any essential contacts in the signalling DDR dimer. This leaves the collagen-bound DS domain as the most likely site of contact between the extracellular regions of the two DDR protomers in the signalling dimer.

An analysis of crystal lattice contacts in the DDR1-3E3 Fab structure led to the fortuitous discovery of functionally important residues near the base of the DS domain, close to the interface with the DS-like domain and distant from the collagen binding site at the top of the DS domain. The patch formed by these residues is the largest concentration of conserved surface residues in the extracellular region of DDRs apart from the collagen binding site, consistent with its essential role in signalling. We think that the conserved patch is involved in mediating protomer contacts in the signalling DDR dimer, either by forming a direct DS-DS interface or by providing a secondary collagen binding site. The latter alternative (which corresponds to the "composite binding site" model discussed previously by Carafoli et al., 2009) is more appealing, as it provides a plausible mechanism whereby collagen could cross-link two DS domains. In solution, the isolated DDR2 DS domain binds a 28-residue collagen peptide with 1:1 stoichiometry (Carafoli et al., 2009). However, inspection of the crystal lattice of this DS-collagen complex reveals that the conserved patch is involved in a lattice contact with the N-terminal glycine-proline-hydroxyproline triplets of the collagen peptide. This intriguing observation may suggest that the conserved patch in the DS domain indeed has weak affinity for collagenous sequences and, therefore, could provide a secondary collagen binding site in dimeric, full-length, DDR.

Whichever interactions are formed between the DS domains and the collagen ligand, they are expected to lead to structural changes within the DDR dimer that are propagated across the cell membrane to result in DDR autophosphorylation (Noordeen et al., 2006). Tight coupling of the extracellular conformational changes to intracellular domain arrangements is difficult to imagine in the DDRs, given their long, and presumably flexible, juxtamembrane regions. Recent studies of the epidermal growth

factor receptor (EGFR) have shown that the conformational coupling across the cell membrane is looser than commonly believed even in a receptor with less extensive juxtamembrane regions (Lu et al., 2010; Mi et al., 2011). However, one important difference is that the transmembrane helices of DDRs have a much higher propensity for self-interactions than that of EGFR (Finger et al., 2009). We propose that the transmembrane helices are largely responsible for constitutive DDR dimerisation (Noordeen et al., 2006), but that collagen-induced interactions involving the DS domains are additionally required for DDR activation.

10 **References for Example 1**

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Example 2: Blocking of collagen-induced DDR1 phosphorylation in cancer cell lines

We set out to show that the antibodies which bind the discoidin-like domain of human
5 DDR1 also block DDR1 activation resulting from collagen binding in cancer cell lines. To
this end, we used the colon carcinoma cell line DLD-1, and the breast cancer cell lines
SKBr-3, MCF-7 and T47D.

Cells were stimulated (or not) with 10 µg/ml collagen (rat tail collagen I, Coll I,) for 90
10 minutes at 37°C in the presence or absence of 10 µg/ml anti-DDR1 mAb 1F10, as
indicated in Figure 12. DDR1 was immunoprecipitated with rabbit anti-DDR1 antibodies
and the immunoprecipitates were analysed by SDS-PAGE and Western blotting. The
blots were probed with anti-phosphotyrosine (anti-PY) mAb 4G10 (upper blots) and
reprobed with anti-DDR1 Abs (lower blots).

15

As can be seen from Figure 12, antibody 1F10, which binds the discoidin-like domain of
human DDR1, blocks collagen-induced autophosphorylation in cancer cell lines.

CLAIMS

1. An antibody that specifically binds to the extracellular domain of human discoidin domain receptor 1 (DDR1) outside the discoidin domain, and which reduces or blocks
5 collagen-induced autophosphorylation of DDR1 without preventing collagen binding to DDR1.
2. An antibody according to Claim 1 that specifically binds to the discoidin-like domain of DDR1.
- 10 3. An antibody according to Claim 1 or 2 that specifically binds to the DDR1 epitope defined by the amino acid residues Ala279, Gln281, Ala282, Ser335, Pro337, Gly340, Arg341, Val342, Ile365 and Asp367.
- 15 4. An antibody according to Claims 1 or 2 that specifically binds to the DDR1 epitope encompassed by the amino acid residues Tyr203 to Tyr209, or to the DDR1 epitope defined by the amino acid residues Met318, Asn321 and Asn325.
- 20 5. An antibody according to any of Claims 1-4, which inhibits growth of a tumour cell.
6. An antibody according to Claim 5, wherein the tumour cell is selected from the group consisting of a breast, colorectal, hepatic, renal, lung, pancreatic, ovarian, prostate, head and neck, gastrointestinal, melanoma, cervical, bladder, glioblastoma,
25 skin, endometrial, stomach, testis, brain, thyroid, non-Hodgkin lymphoma, Hodgkin lymphoma, and nasal NK/T-cell lymphoma, tumour cell.
7. An antibody according to any of Claims 1-6, which reduces the frequency of cancer stem cells in a tumour.
- 30 8. An antibody that specifically binds to the discoidin-like domain of human DDR1, wherein the antibody comprises:
 - a heavy chain CDR1 comprising the amino acid sequence SGYTFSISW,
 - a heavy chain CDR2 comprising the amino acid sequence YPSGGY, and/or
 - 35 a heavy chain CDR3 comprising the amino acid sequence RGYGHLEY,or a variant of any of these sequences comprising 1, 2 or 3 amino acid substitutions.

9. An antibody according to Claim 8 which further comprises:
 a light chain CDR1 comprising the amino acid sequence SSSVTF,
 a light chain CDR2 comprising the amino acid sequence YLTSN, and/or
 a light chain CDR3 comprising the amino acid sequence QWSSNPYT,
 5 or a variant of any of these sequences comprising 1, 2 or 3 amino acid substitutions.
10. An antibody that specifically binds to the discoidin-like domain of human DDR1,
 wherein the antibody comprises:
 a light chain CDR1 comprising the amino acid sequence SSSVTF,
 10 a light chain CDR2 comprising the amino acid sequence YLTSN, and/or
 a light chain CDR3 comprising the amino acid sequence QWSSNPYT,
 or a variant of any of these sequences comprising 1, 2 or 3 amino acid substitutions..
11. An antibody that specifically binds to the discoidin-like domain of human DDR1,
 15 wherein the antibody comprises:
 (a) a heavy chain CDR1 comprising the amino acid sequence SGYTFSISW,
 a heavy chain CDR2 comprising the amino acid sequence YPSGGY, and/or
 a heavy chain CDR3 comprising the amino acid sequence RGYGHLEY, and
 (b) a light chain CDR1 comprising the amino acid sequence SSSVTF,
 20 a light chain CDR2 comprising the amino acid sequence YLTSN, and/or
 a light chain CDR3 comprising the amino acid sequence QWSSNPYT,
 or a variant of any of these sequences comprising 1, 2 or 3 amino acid substitutions.
12. An antibody that specifically binds to the discoidin-like domain of human DDR1,
 25 wherein the antibody comprises:
 (a) a heavy chain variable region sequence
 QVQLQESGAELVRPGASVKLSCKASGYTFSISWINWVKQRPQGQLEWIGNIYPSGGYTNY
 NQKFQDKATLTVDKSSNTAYIQLSSPTSEDSAVYYCTRGYGHLDYWGQGTLLTVSA; and/or
 (b) a light chain variable region sequence,
 30 DIQLTQSPALMSASPGEKVTMTCSASSSVTFMYWYQKPRSSPKPWIYLTSLNLSGVPAR
 FSGSGSGTSLTISMEAEADAATYYCQQWSSNPYTFGGGTKLEIK,
 or a variant of any of these sequences comprising 1, 2, 3, 4 or 5 amino acid
 substitutions.
- 35 13. The antibody according to any of Claims 8-12, wherein the amino acid
 substitutions are conservative amino acid substitutions.

14. An antibody that comprises:

(a) a heavy chain sequence

QVQLQESGAELVRPGASVKLSCKASGYTFSISWINWVKQRPQGQLEWIGNIYPSGGYTNY
 5 NQKFQDKATLTVDKSSNTAYIQLSSPTSEDSAVYYICTRGYGHLDYWGQGTSLVTVSAAKTT
 PPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTL
 SSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVP; and

(b) a kappa light chain sequence

DIQLTQSPALMSASPGEKVTMTCSASSSVTFMYWYQQKPRSSPKPWIYLTSLNLAGVPAR
 10 FSGSGSGTSSYSLTISSMEAEDAATYYCQQWSSNPYTFGGGTKLEIKRADAAPTVSIFPPS
 SEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDYSTYSMSSTLTLL
 TKDEYERHNSYTCEATHKSTSTSPIVKSFNRNEC.

15. An antibody that competes with an antibody according to Claim 14 for specific
 15 binding to the discoidin-like domain of human DDR1.

16. A binding moiety that specifically binds to the human DDR1 epitope defined by
 the amino acid residues Ala279, Gln281, Ala282, Ser335, Pro337, Gly340, Arg341,
 Val342, Ile365 and Asp367.

20

17. A binding moiety that specifically binds to the human DDR1 epitope
 encompassed by the amino acid residues Tyr203 to Tyr209, or to the human DDR1
 epitope defined by the amino acid residues Met318, Asn321 and Asn325.

25 18. A binding moiety according to Claim 16 or 17 which reduces or blocks collagen-
 induced autophosphorylation of DDR1 without preventing collagen binding to DDR1.

19. A binding moiety according to any of Claims 16-18 which is an antibody, an
 antibody mimic (for example, based upon a non-antibody scaffold), an RNA aptamer, a
 30 small molecule or a CovX-body.

20. An antibody according to any of Claims 1-15 or 19, which is an IgA, IgD, IgE, IgG
 or IgM antibody, such as an IgG1, IgG2 or IgG4 antibody.

35 21. An antibody according to any of Claims 1-15 or 19-20 wherein the antibody is an
 antigen-binding fragment of an antibody, for example selected from the group consisting

of: Fv fragments (*e.g.* single chain Fv and disulphide-bonded Fv); Fab-like fragments (*e.g.* Fab fragments, Fab' fragments and F(ab)₂ fragments); and domain antibodies.

22. An antibody according to Claim 21 wherein the antigen-binding fragment is an
5 scFv.

23. An antibody according to any of Claims 1-15 or 19-22 wherein the antibody is a recombinant antibody.

10 24. An antibody according to any of Claims 1-15 or 19-23 wherein the antibody is a monoclonal antibody.

25. An antibody according to any of Claims 1-15 or 19-24 wherein the antibody is human or humanised.
15

26. An antibody or a binding moiety according to any of the preceding claims which does not specifically bind to human discoidin domain receptor 2 (DDR2).

27. An antibody or a binding moiety according to any of the preceding claims which is
20 in an isolated and/or purified form.

28. A polynucleotide encoding an antibody or a binding moiety according to any of the preceding claims, provided that the binding moiety is a polypeptide.

25 29. An expression vector comprising the polynucleotide of Claim 28.

30. A host cell comprising the polynucleotide of Claim 28 or the expression vector of Claim 29.

30 31. A pharmaceutical composition comprising an antibody or a binding moiety according to any of Claims 1-27 and a pharmaceutically acceptable diluent, carrier or excipient.

32. A pharmaceutical composition comprising an antibody or a binding moiety
35 according to any of Claims 1-27, and at least one additional anti-cancer agent, and a pharmaceutically acceptable diluent, carrier or excipient.

33. A kit of parts comprising an antibody or a binding moiety according to any of Claims 1-27 and at least one additional anti-cancer agent.
34. An antibody or a binding moiety according to any of Claims 1-27 and, optionally,
5 at least one additional anti-cancer agent, for use in medicine.
35. A method of inhibiting activity of DDR1 on a cell, the method comprising contacting the cell with an effective amount of an antibody or a binding moiety according to any of Claims 1-27.
- 10 36. The method of Claim 35 wherein the cell is a tumour cell.
37. The method of Claim 35 or 36 which is performed on cells *in vitro*.
- 15 38. A method of inhibiting growth of a tumour in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody or a binding moiety according to any of Claims 1-27, or a pharmaceutical composition according to Claim 31-32, and, optionally, at least one additional anti-cancer agent.
- 20 39. A method of reducing tumorigenicity of a tumour that comprises cancer stem cells in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody or a binding moiety according to any of Claims 1-27, or a pharmaceutical composition according to Claim 31-32, and, optionally, at least one additional anti-cancer agent, wherein the frequency of cancer stem cells in the tumour is
25 reduced by the administration of the antibody or the binding moiety.
40. The method of Claim 38 or 39, wherein the tumour is a breast tumour, colorectal tumour, hepatic tumour, kidney tumour, liver tumour, lung tumour, pancreatic tumour, gastrointestinal tumour, melanoma, ovarian tumour, prostate tumour, cervical tumour,
30 bladder tumour, glioblastoma, head and neck tumour, skin tumour, endometrial tumour, stomach tumour, testis tumour, brain tumour or thyroid tumour.
41. A method of treating cancer in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody or a binding
35 moiety according to any of Claims 1-27, or a pharmaceutical composition according to Claim 31-32, and, optionally, at least one additional anti-cancer agent.

42. The method of Claim 41, wherein the cancer is a breast cancer, colorectal cancer, hepatic cancer, kidney cancer, liver cancer, lung cancer, pancreatic cancer, gastrointestinal cancer, melanoma, ovarian cancer, prostate cancer, cervical cancer, bladder cancer, glioblastoma, head and neck cancer, skin cancer, endometrial cancer, stomach cancer, testis cancer, brain cancer or thyroid cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, and nasal NK/T-cell lymphoma.

43. An antibody or a binding moiety according to any of Claims 1-27, or a pharmaceutical composition according to Claim 31-32, and, optionally, at least one additional anti-cancer agent, for use in inhibiting growth of a tumour in a human patient, or for use in reducing the tumorigenicity of a tumour that comprises cancer stem cells in a human patient, or for use in treating cancer in a human patient.

44. Use of an antibody or a binding moiety according to any of Claims 1-27, or a pharmaceutical composition according to Claim 31-32, and, optionally, at least one additional anti-cancer agent, in the preparation of a medicament for inhibiting growth of a tumour in a human patient, or for use in reducing the tumorigenicity of a tumour that comprises cancer stem cells in a human patient, or for use in treating cancer in a human patient.

45. Use of an antibody or a binding moiety according to any of Claims 1-27, or a pharmaceutical composition according to Claim 31, in the preparation of a medicament for inhibiting growth of a tumour in a human patient, or for use in reducing the tumorigenicity of a tumour that comprises cancer stem cells in a human patient, or for use in treating cancer in a human patient, wherein the patient is one who is being administered at least one additional anti-cancer agent.

46. An antibody for use according to Claim 43, or the use of Claim 44 or 45, wherein the tumour or cancer is a breast, colorectal, hepatic, kidney, liver, lung, pancreatic, gastrointestinal, melanoma, ovarian, prostate, cervical, bladder, glioblastoma or head and neck tumour or cancer, endometrial cancer, stomach cancer, testis cancer, or thyroid cancer.

47. A method of treating an inflammatory disorder, a fibrotic disorder or atherosclerosis in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody or a binding moiety according to any of Claims 1-27, or a pharmaceutical composition according to Claim 31, and, optionally, at

least one additional agent suitable for treating the inflammatory disorder, a fibrotic disorder or atherosclerosis.

48. An antibody or a binding moiety according to any of Claims 1-27, or a pharmaceutical composition according to Claim 31, for use in for use in treating an inflammatory disorder, a fibrotic disorder or atherosclerosis in a human patient, optionally, together with at least one additional agent suitable for treating the inflammatory disorder, fibrotic disorder or atherosclerosis.

49. Use of an antibody or a binding moiety according to any of Claims 1-27, in the preparation of a medicament for treating an inflammatory disorder, a fibrotic disorder or atherosclerosis in a human patient, wherein, optionally, the patient is one who is administered at least one additional agent suitable for treating the inflammatory disorder, fibrotic disorder or atherosclerosis.

50. Use of an antibody or a binding moiety according to any of Claims 1-27, and at least one additional agent suitable for treating an inflammatory disorder, fibrotic disorder or atherosclerosis, in the preparation of a medicament for treating an inflammatory disorder, a fibrotic disorder or atherosclerosis in a human patient.

51. A method or a use according to any of Claims 47-50 wherein the inflammatory disorder is inflammation associated with atherosclerosis, inflammation associated with chronic kidney disease or inflammation associated with lung fibrosis.

52. A method or a use according to any of Claims 47-50 wherein the fibrotic disorder is lung fibrosis, liver fibrosis, kidney fibrosis, or fibrosis associated with atherosclerosis.

53. A method or a use according to any of Claims 47-50 wherein the disorder to be treated is atherosclerosis

54. A compound comprising an antibody or a binding moiety according to any of Claims 1-27 and a detectable moiety.

55. A compound according to Claim 54 wherein the detectable moiety comprises a radioactive atom.

56. An agent according to Claim 55 wherein the radioactive atom is selected from the group consisting of: technetium-99; technetium-99m; iodine-123; iodine-124; iodine-131; indium-111; fluorine-18; fluorine-19; carbon-11; carbon-13; copper-64; nitrogen-13; nitrogen-15; oxygen-15; oxygen-17; arsenic-72; gadolinium; manganese; iron; deuterium; tritium; yttrium-86; zirconium-89.

57. A compound comprising an antibody or a binding moiety according to any of Claims 1-27 and a cytotoxic moiety.

58. A compound according to Claim 57 wherein the cytotoxic moiety comprises a radioactive atom.

59. A compound according to Claim 58 wherein the radioactive atom is selected from the group consisting of: iodine-123; iodine-125; iodine-131; indium-111; bromine-77; copper-67; arsenic-77; astatine-211; actinium-225; bismuth-212; bismuth-213; lutetium-177; holmium-166; phosphorous-33; platinum-193; platinum-195; rhenium-186; rhenium-188; strontium-89; yttrium-90.

60. A compound according to Claim 57 wherein the cytotoxic moiety comprises a drug selected from the group consisting of: an alkylating agent (such as cisplatin, carboplatin); an antimetabolite (such as azathioprine, methotrexate); an antimitotic drug (such as vincristine); a topoisomerase inhibitor (such as doxorubicine, etoposide); a toxin (such as calicheamicin).

61. A method of identifying an agent that inhibits activity of human DDR1, or a lead compound for identifying an agent that inhibits activity of human DDR1, the method comprising:

assaying a test agent for binding to:

- (i) the human DDR1 epitope defined by the amino acid residues Ala279, Gln281, Ala282, Ser335, Pro337, Gly340, Arg341, Val342, Ile365 and Asp367, or
- (ii) the human DDR1 epitope encompassed by the amino acid residues Tyr203 to Tyr209, or
- (ii) the human DDR1 epitope defined by the amino acid residues Met318, Asn321 and Asn325,

wherein a test agent that binds to one of the said DDR1 epitopes may be an agent that inhibits activity of human DDR1, or a lead compound for identifying an agent that inhibits activity of human DDR1.

5 62. A method according to Claim 61, wherein the assaying step comprises predicting whether the test agent binds to the said DDR1 epitope by molecular modelling *in silico*.

63. A method according to Claim 61 or 62 wherein the assaying step comprises determining whether the test agent competes with an antibody according to Claim 14 for
10 specific binding to the discoidin-like domain of human DDR1.

64. A method according to any of Claims 61-63, wherein the test agent is an antibody, an antibody mimic (for example, based upon a non-antibody scaffold), an RNA aptamer, a small molecule, a CovX-body, a natural product, a polypeptide, or a nucleic
15 acid

65. A method according to Claim 64 wherein the antibody is an antigen-binding fragment of an antibody, for example selected from the group consisting of: Fv fragments (e.g. single chain Fv and disulphide-bonded Fv); Fab-like fragments (e.g. Fab fragments, Fab' fragments and F(ab)₂ fragments); and domain antibodies.
20

66. A method according to Claims 61-65 further comprising the step of determining whether the test agent reduces collagen-induced autophosphorylation of DDR1.

25 67. A method according to any of Claims 61-66, wherein an agent identified as a result of the method is modified and retested.

68. A method according to any of Claims 61-67, wherein an agent having or expected to have similar properties to an agent identified as a result of the method is
30 tested.

69. A method according to any of Claims 61-68, wherein an agent identified as a result of the method is tested for efficacy in a cell model and/or an animal model of a condition selected from cancer, an inflammatory disorder, a fibrotic disorder, or
35 atherosclerosis.

70 A method according to any of Claims 61-69, wherein an agent identified as a result of the method is further tested for efficacy and safety in a clinical trial of a condition selected from cancer, an inflammatory disorder, a fibrotic disorder, or atherosclerosis.

5 71. A method according to any of Claims 61-69, wherein an agent identified as a result of the method is synthesised.

72. A method according to any of Claims 63-71, wherein an agent identified as a result of the method is packaged and presented for use in treating a condition selected
10 from cancer, an inflammatory disorder, a fibrotic disorder, or atherosclerosis.

73. An antibody substantially as described herein with reference to the description.

74. A binding moiety substantially as described herein with reference to the
15 description.

75. An antibody for use in medicine substantially as described herein with reference to the description.

20 76. A binding moiety for use in medicine substantially as described herein with reference to the description.

77. A compound for use in medicine substantially as described herein with reference to the description.
25

78. Use of an antibody substantially as described herein with reference to the description.

79. Use of a binding moiety substantially as described herein with reference to the
30 description.

80. A pharmaceutical composition substantially as described herein with reference to the description

35 81. A kit substantially as defined herein with reference to the description.

82. A method of identifying an agent substantially as described herein with reference to the description.

Figure 1

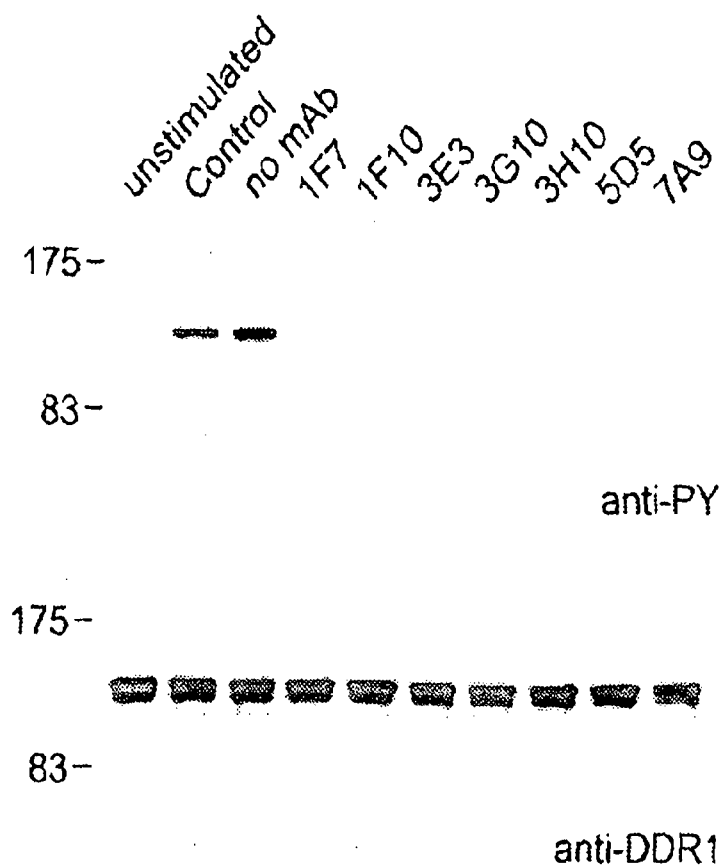


Figure 2

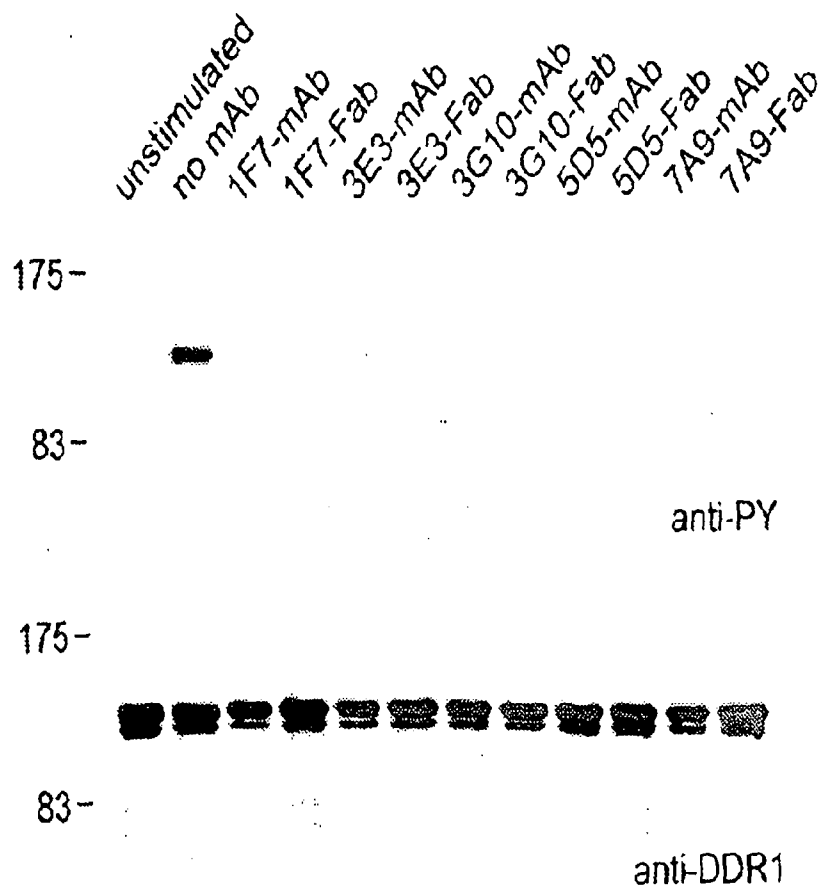


Figure 3

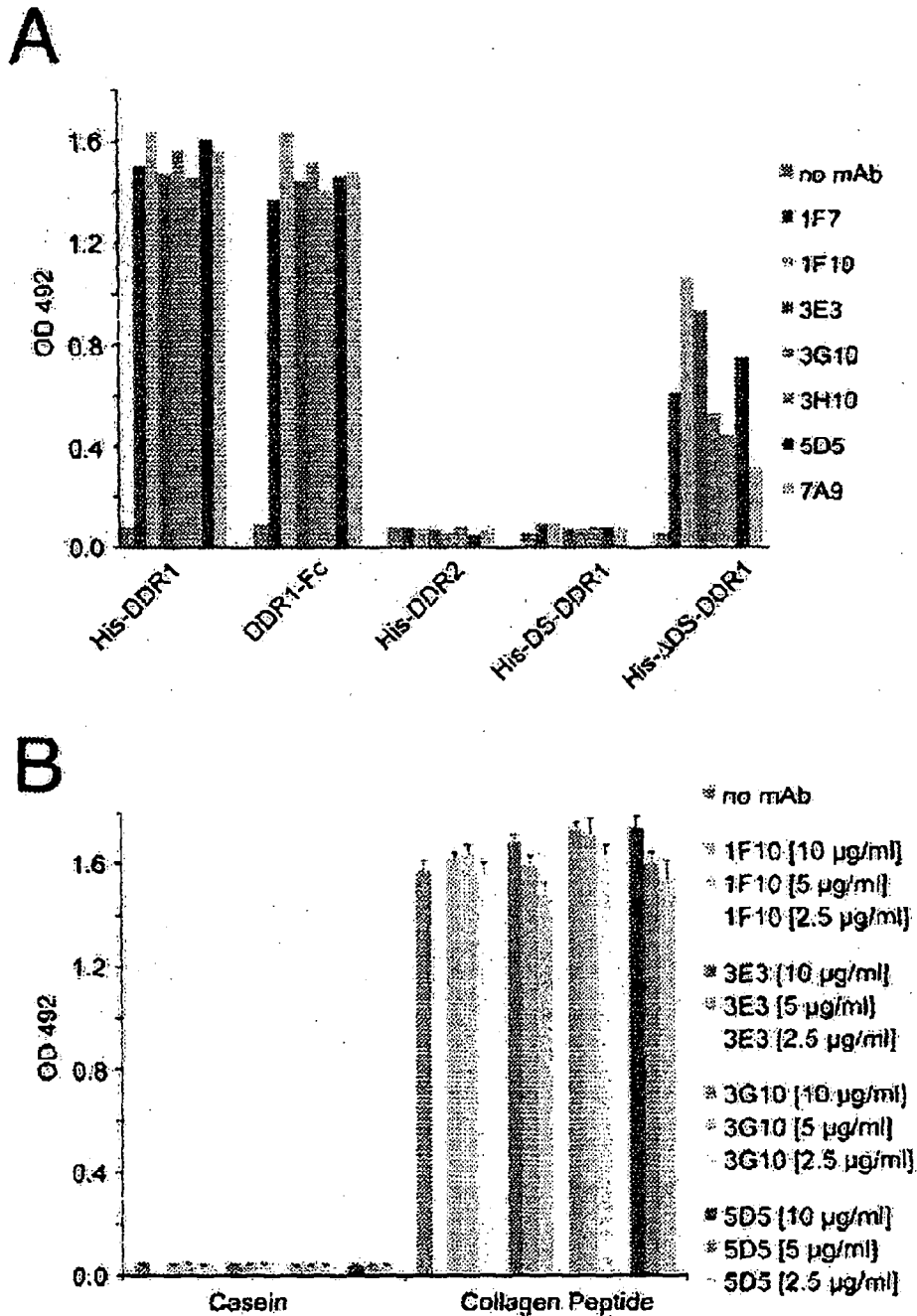


Figure 4

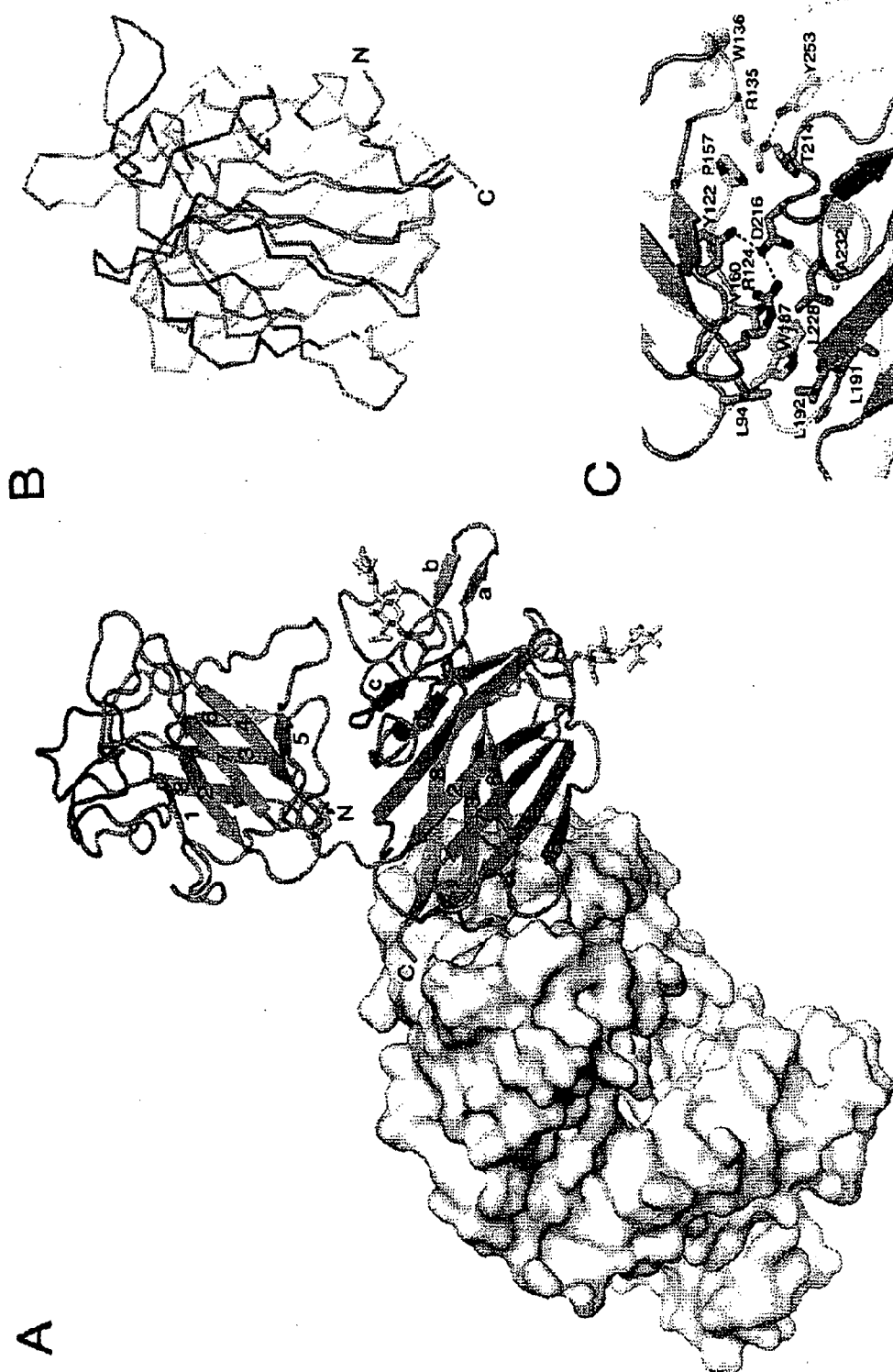


Figure 5 (Part 1 of 2)

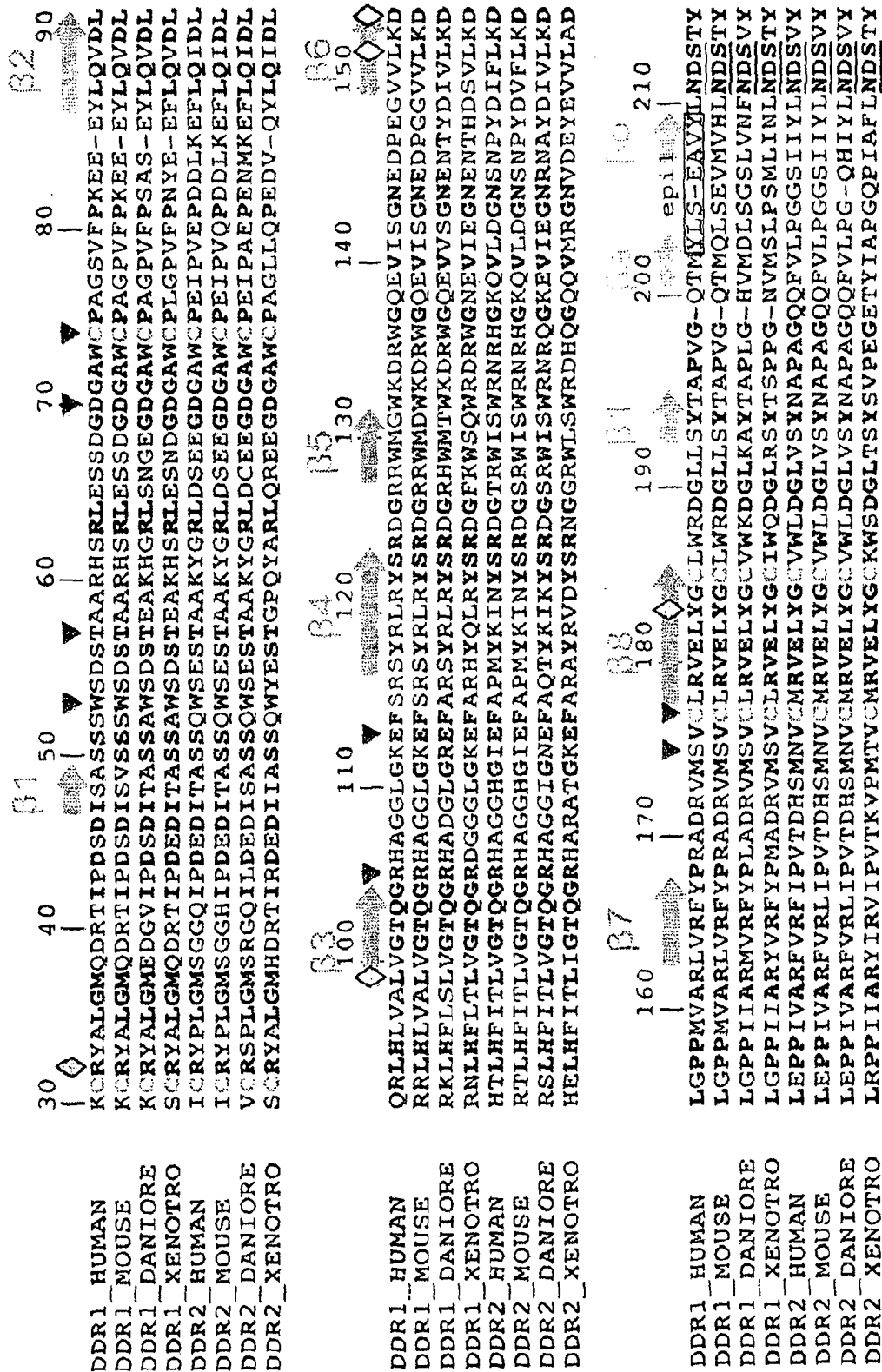


Figure 6

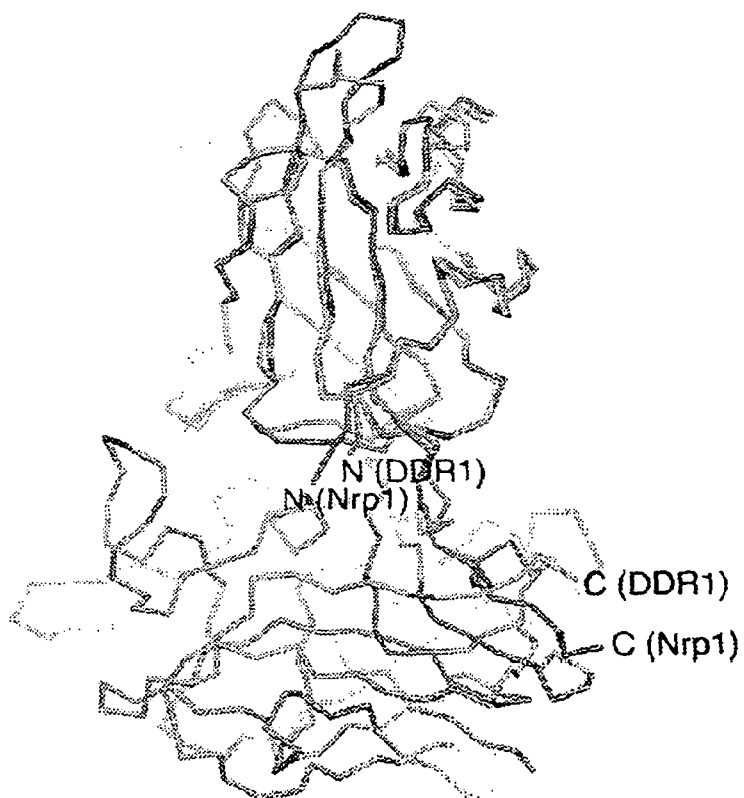
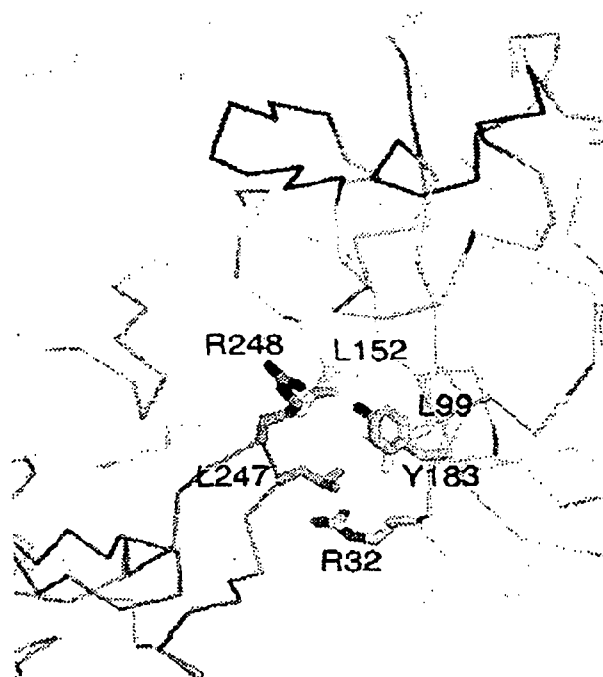


Figure 7

A



B

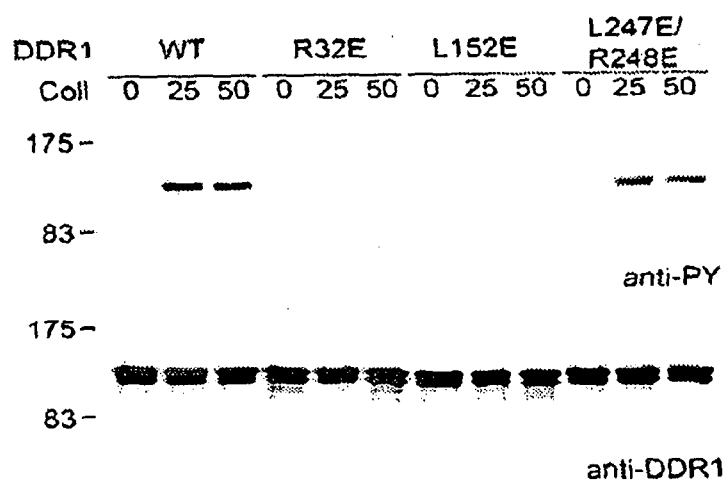


Figure 8

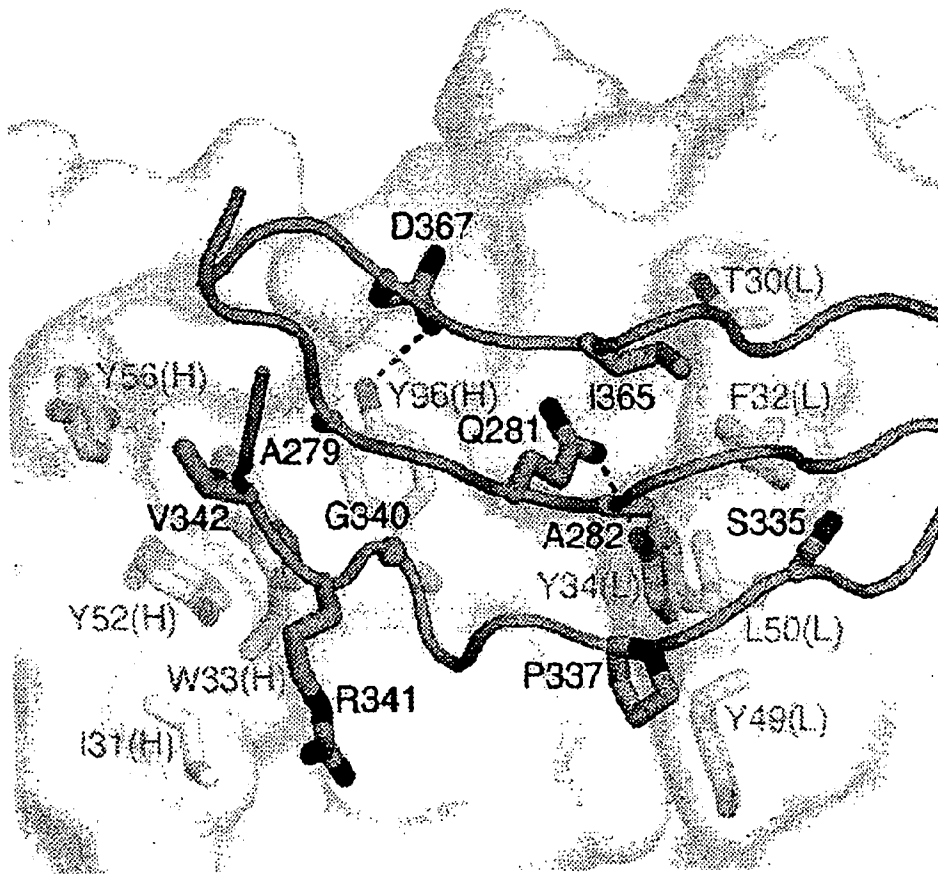


Figure 9 (Part 1 of 2)

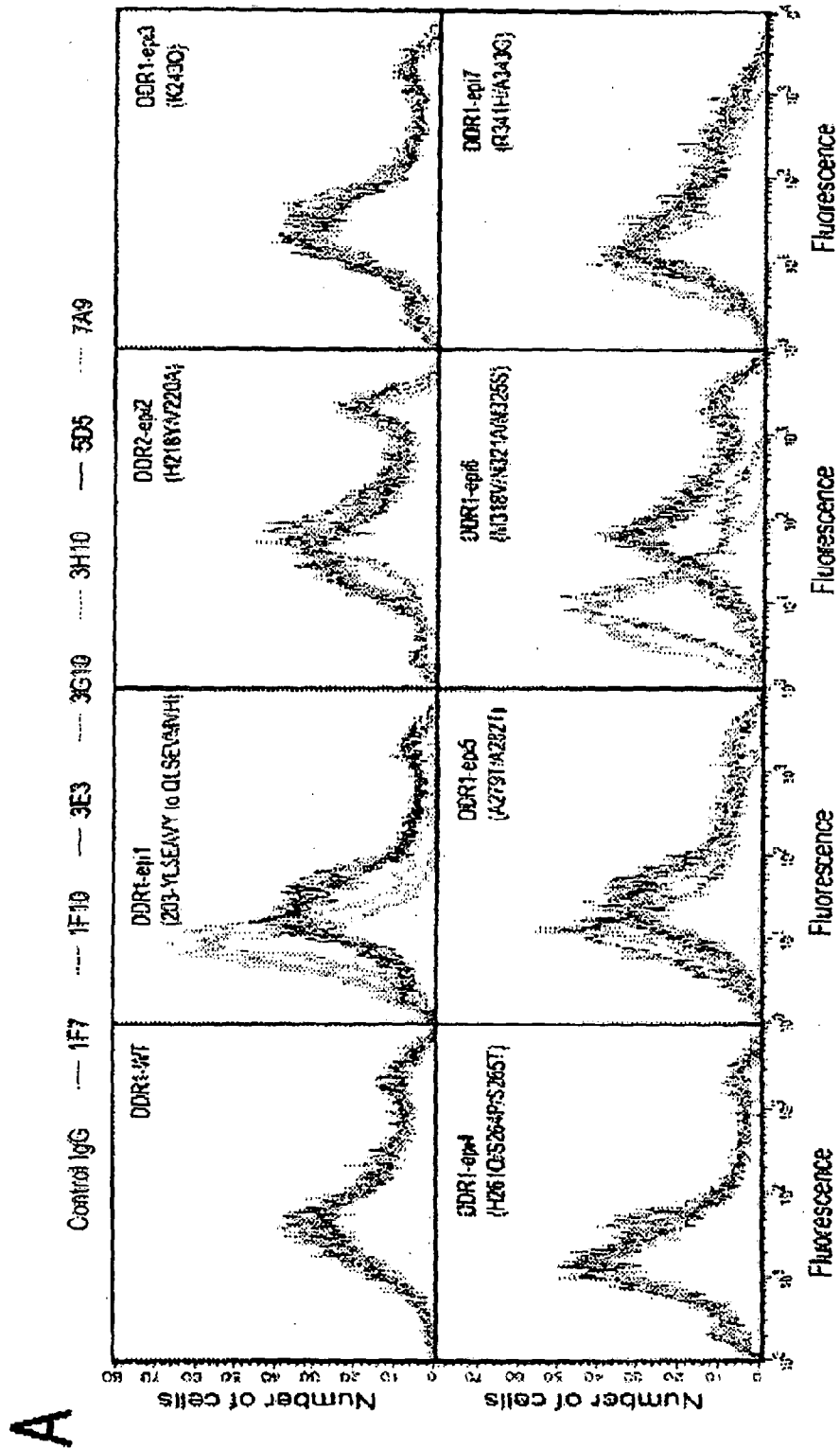


Figure 9 (Part 2 of 2)

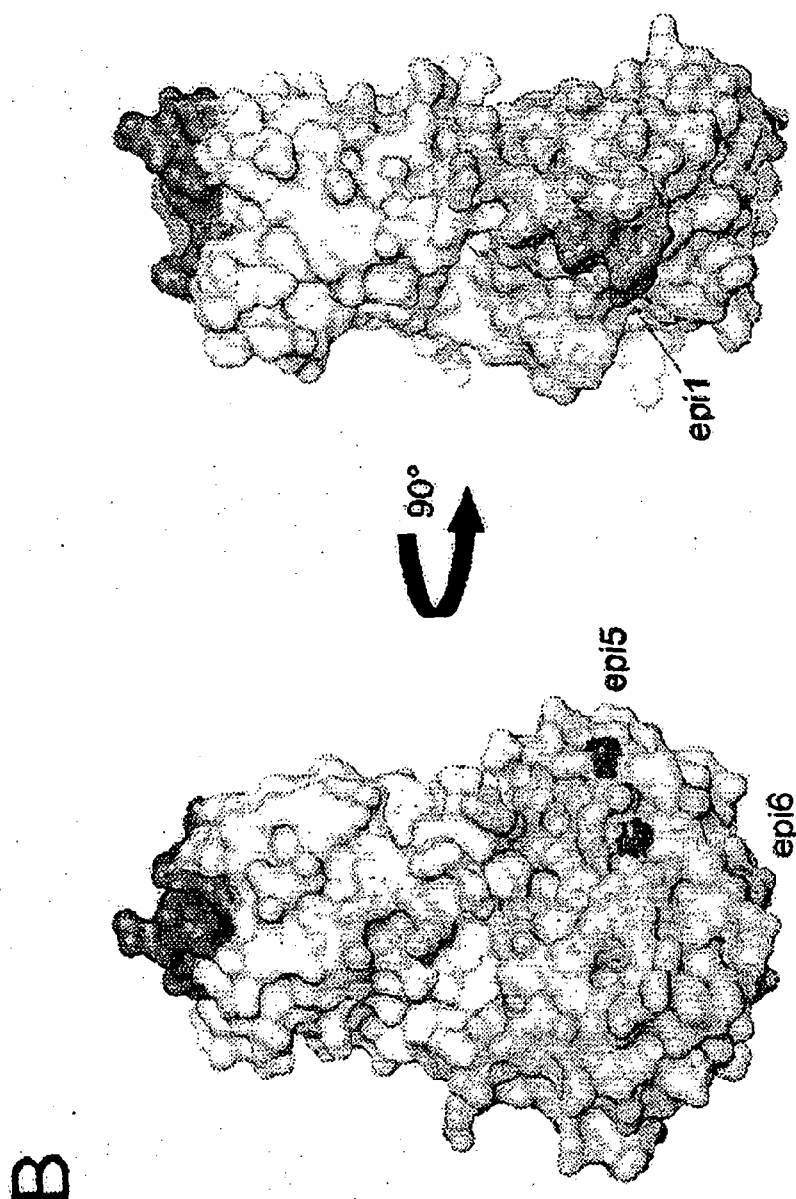


Figure 10

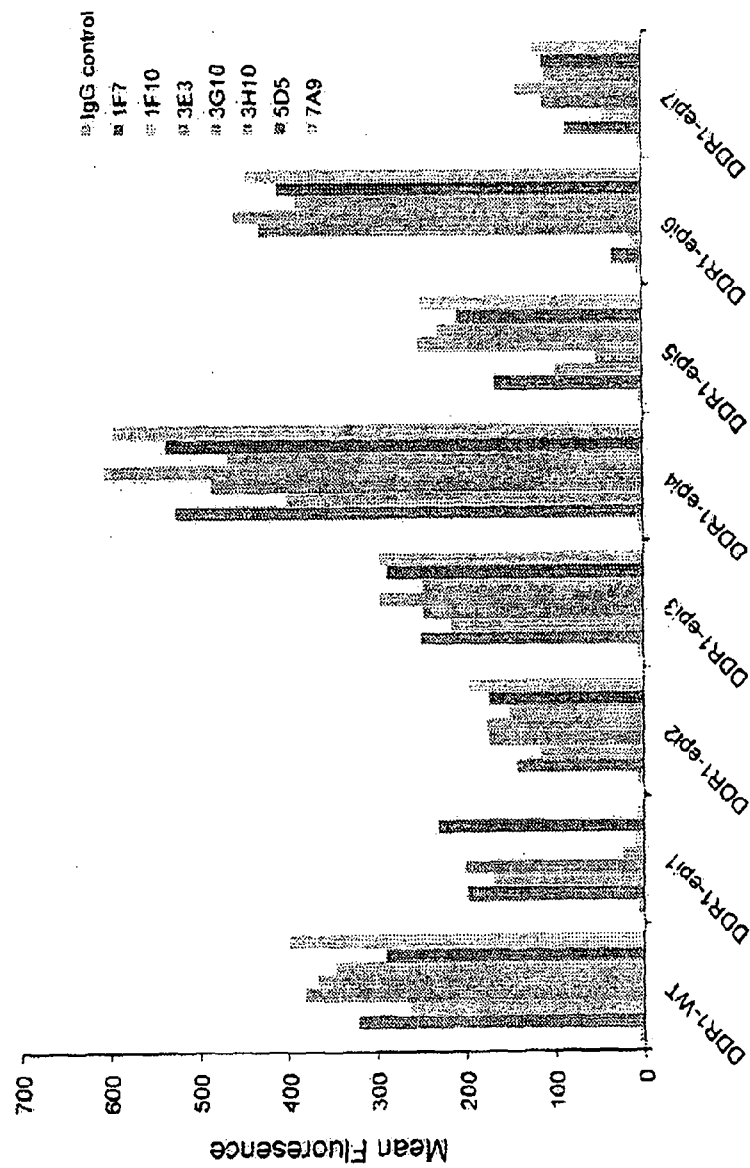


Figure 11

3E3_H

QVQLQESGAELVRPGASVKLSCKASGYTFSISWINWVKQRPQGLEWIGNIYPSGGYTNY

3E3_H

NQKFKDKATLTVDKSSNTAYIQLSSPTSEDSAVYYCTRGYGHLDYWGQGLVTVSAAKTT

3E3_H

PPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTL

3E3_H

SSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVP

light chain (kappa)

3E3_L

DIQLTQSPALMSASPGEKVTMTCSASSSVTFMYWYQQKPRSSPKPWIYLTSNLASGVPAR

3E3_L

FSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPYTFGGGTKLEIKRADAAAPTVSIFPPS

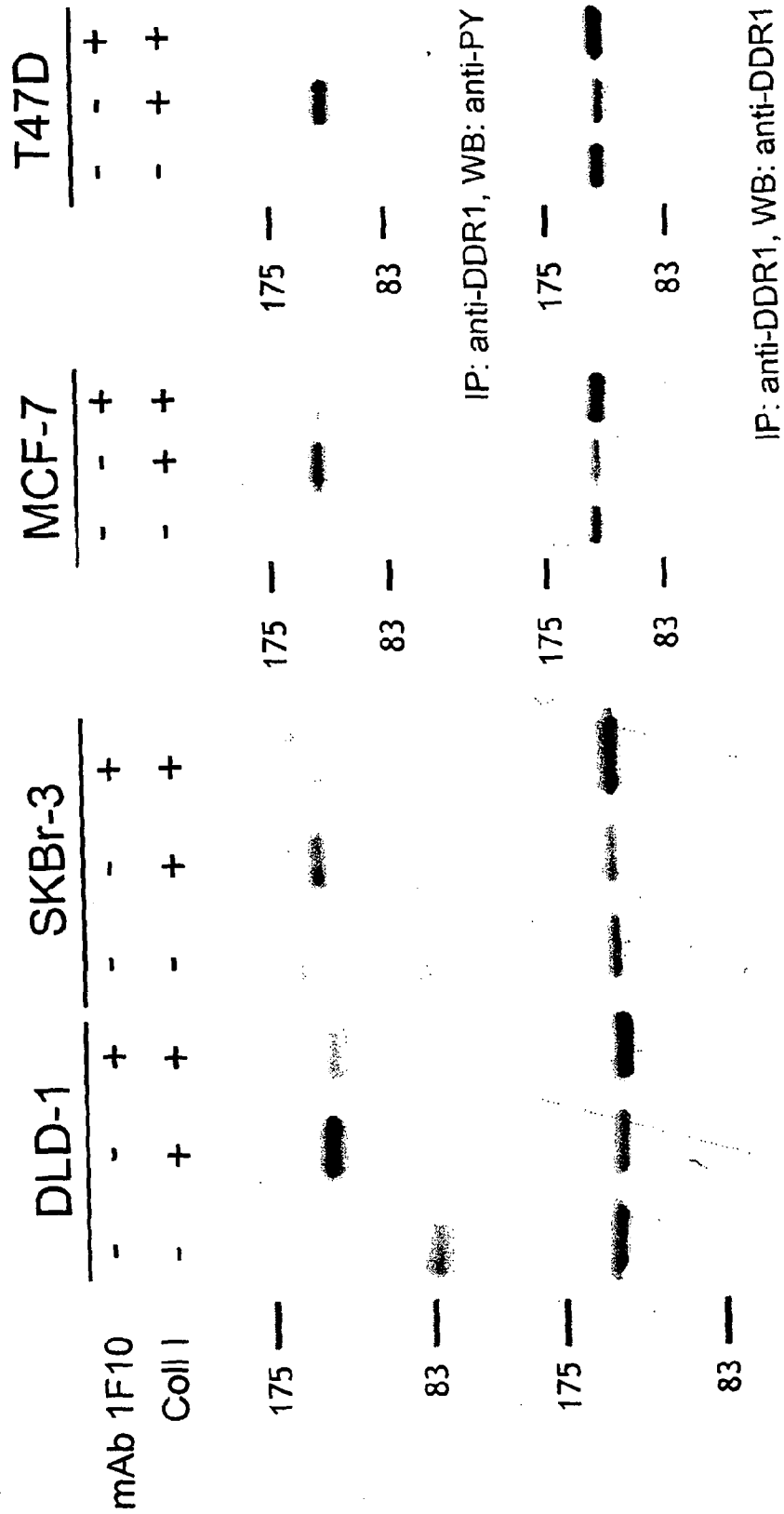
3E3_L

SEQLTSGGASVVCFLNMFYPKDINVKKIDGSERQNGVLNSWTDQDSKDYSTYSMSSTLTL

3E3_L

TKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

Figure 12



INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2012/052219

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/28 C07K16/30
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2012/146585 A1 (SANOFI SA [FR]; BREITSCHOPF KRISTIN [DE]; DE HOOP MELTSJE [DE]) 1 November 2012 (2012-11-01) examples 4,8	1-82
X	WO 2010/019702 A2 (ONCOMED PHARM INC [US]; GURNEY AUSTIN L [US]; VAN DER HORST EDWARD THE) 18 February 2010 (2010-02-18) cited in the application example 1 paragraphs [0066], [0102], [0034], [0107], [0111] - [0112]; claims 6-7, 26, 37-41, 42-45, 59-63; examples 3-6	1,2, 5-13, 15-82

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 November 2012

Date of mailing of the international search report

20/12/2012

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2012/052219

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012146585 A1	01-11-2012	EP 2518157 A1 WO 2012146585 A1	31-10-2012 01-11-2012
WO 2010019702 A2	18-02-2010	US 2011287011 A1 WO 2010019702 A2	24-11-2011 18-02-2010