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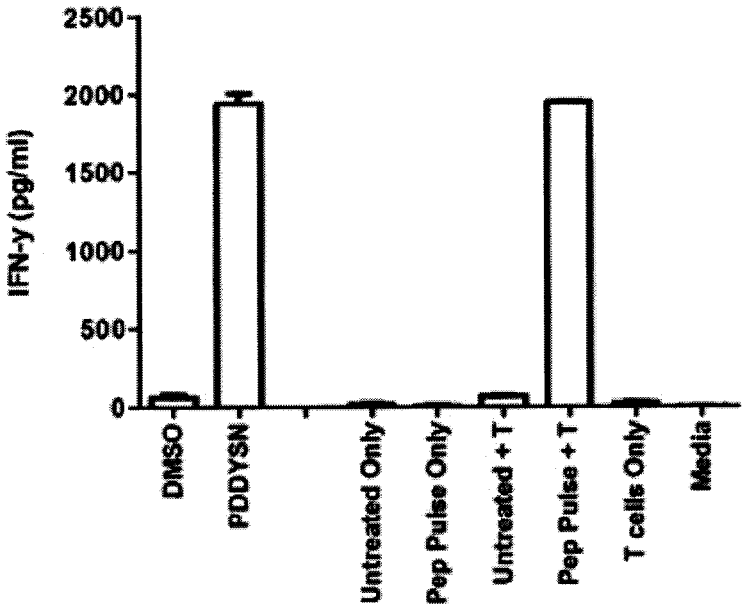
(54) Title: AGENTS AND METHODS

(57) Abstract: The invention provides an agent comprising: (i) a T cell antigen, and (ii) a binding partner for any of CD22, CD23, CD30, CD74, CD70, CD43, CD44, CD47, CD54, CD58, CD62L, CD95, HLA-DR, CD59, CD55, wherein, following binding of the agent to a cell that expresses any of CD22, CD23, CD30, CD74, CD70, CD43, CD44, CD47, CD54, CD58, CD62L, CD95, HLA-DR, CD59, CD55, the agent is internalised and the T cell antigen is presented on the surface of the cell in a form that can be recognised by a T cell.

Figure 2

A

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# AGENTS AND METHODS

The present invention relates to immunotherapeutic agents. In particular, it relates to agents that can be used to prevent or treat a condition characterised by the presence of unwanted cells, such as tumours or other disease causing cells.

Immunotherapeutic strategies for targeting malignant disease are an active area of translational clinical research, and have been for several decades. The current models dictate that cancer represents either a functional or constitutional immunodeficiency which can be treated with immunotherapeutic manipulation of the host. These efforts can be broadly classified into two groups. The first serves to augment or support endogenous anti-tumour immunity through measures such as vaccination, cytokine support (IL-2, IFNγ) or reducing immunosuppressant environment (ipilimumab) whilst the second seeks to restore an absolute deficiency with components of a functional immune response (passive immunotherapy with antibodies, TCR transfer, Stem Cell Transplantation and adoptive immunotherapy). These approaches are unified by the argument that a highly effective functional anti-tumour immune response is indeed possible. Although irrefutable evidence exists for an effective anti-tumour immune response in some cases, this central pillar of tumour immunology is overwhelmingly countered by the current clinical reality that despite great efforts, no effective immunotherapeutics are available for the majority of patients with cancer. Almost all cancer vaccination trials have provided negative results, with those providing positive data most frequently demonstrating a small effect. The reality is that therapeutic antibodies, with a few exceptions, offer very modest clinical benefit in the area of oncology.

If a therapeutic strategy could be developed which can efficiently molecularly re-direct an endogenous anti-viral immune response to instead target malignant tissue, this may afford a new powerful and safe approach to treat malignant disease.

The majority of cytotoxic therapeutic antibodies rely on immunological effector mechanisms to deliver their anti-cancer effect such as complement dependent cytotoxicity (CDC) and Antibody Dependent Cellular Cytotoxicity (ADCC). Importantly, all cells (both healthy and malignant) have numerous mechanisms to limit attack by the immune response to avert autoimmunity. This is evident in the context of autoimmune disease where high levels of tissue-reactive antibodies, which although frequently evoke organ inflammation, rarely induce complete organ destruction. Indeed, autoimmune

diseases where complete tissue destruction is observed, such as diabetes mellitus, are known to be dependent on CTL responses rather than antibody-directed mechanisms.

To improve upon the poor efficacy of therapeutic antibodies, immunoconjugates (radionuclides/ toxins) and engineered antibodies which better engage with the cytotoxic effector mechanisms (e.g. glycoengineering) have been used. However clinical trials of such agents remain largely disappointing and are plagued by toxicity. One example is antibody-drug conjugates (ADCs) that have been developed to selectively target anti-tumour agents to tumours (see US 5,773,001; US 5,767,285; US 5,739,116; US 5,693,762; US 5,585,089; US 2006/0088522; US 2011/0008840; US 7,659,241; Hughes (2010) *Nat Drug Discov* 9: 665, Lash (2010); *In vivo: The Business & Medicine Report* 32-38; Mahato *et al* (2011) *Adv Drug Deliv Rev* **63**: 659; Jeffrey *et al* (2006) *BMCL* **16**: 358; *Drugs R D* 11(1): 85-95). ADCs generally comprise a monoclonal antibody against a target present on a tumour cell, a cytotoxic drug, and a linker that attaches the antibody to the drug. However, only a few ADCs are currently in the late stage of clinical development, and of those that are, clinical success has proven elusive.

Thus, there remains a demand for more effective immunotherapeutic agents with greater efficacy and lower toxicity.

The agents of the invention are an example of re-directed immunotherapy. This refers to the concept of re-directing an existing immune response that normally target cells harbouring foreign antigens, to target unwanted cells in conditions such as cancer. The concept requires the presentation of marker antigens on unwanted cells such that they become a target for immune cells.

WO 95/17212 describes conjugates consisting of peptidic T cell antigens and cell binding partners and their use in re-directed immunotherapy. The conjugates are said to be internalised into target cells following binding of the binding partner to surface receptors, and the T cell antigen is processed from the conjugate and expressed on the cell surface in the form of a complex with MHC molecules. Recognition of the complex by a T cell receptor induces a cytotoxic T cell response against the target cells. However, which binding partners enable internalisation and hence subsequent presentation of the T cell antigen, and which do not, cannot be predicted from WO 95/17212. The only receptors shown to present peptide antigens effectively were antigen receptors of B cells, whose normal role is to bind to and internalise antigen for presentation to helper T cells.

However, WO 95/17212 offers no guidance on which other receptors, if any, are guaranteed to provide the same result.

Surprisingly and unexpectedly, the inventors have now identified particular antigens that have utility in re-directed immunotherapy, namely CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95. Targeting each of these antigens with viral derived peptides led to increased activation of virus-specific T cells *in vitro*, indicating that, surprisingly, each of the antigens enables internalisation of the peptides and subsequent presentation on the cell surface.

CD70 (*TNFSF7*) is a member of the tumour necrosis factor (TNF) superfamily. It is a type II integral membrane protein and a ligand for CD27. The protein is transiently expressed in antigen-activated T and B lymphocytes and its interaction with CD27 regulates T- and B-cell functions. In particular, the protein acts to control death, survival and co-stimulation of target cells. Although CD70 is expressed by limited subsets of normal lymphocytes and dendritic cells, it is aberrantly expressed by a broad range of hematologic malignancies and some solid tumours.

CD74 is an MHC class II chaperone and functions as a membrane receptor for the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) on immune cells. MIF binding to CD74 activates downstream signalling through the MAPK and Akt pathways and promotes cell proliferation and survival. Beside expression by immune cells, CD74 overexpression has been observed in several non-CNS cancers, and CD74 expression in these tumours is generally associated with aggressive behaviour and poor patient prognosis.

CD22 is a molecule belonging to the SIGLEC family of lectins which specifically binds sialic acid with an immunoglobulin (Ig) domain located at its N-terminus. It is found on the surface of mature B cells and to a lesser extent on some immature B cells. It is thought that CD22 is a regulatory molecule that prevents the overactivation of the immune system and the development of autoimmune diseases. It is present on many B cell malignancies including chronic lymphocytic leukaemia and non-Hodgkin's lymphoma.

HLA-DR MHC class II cell surface receptor encoded by the human leukocyte antigen complex on chromosome 6. The complex of HLA-DR and its ligand, a peptide of 9 amino acids in length or longer, constitutes a ligand for the T-cell receptor (TCR). HLA-

DR molecules are upregulated in response to signalling. HLA-DR is found on various cancers including colorectal carcinoma where a increased HLA-DR expression relates to better prognostic outcome.

CD23 is the "low-affinity" receptor for IgE, an antibody isotype involved in allergy and resistance to parasites, and is important in regulation of IgE levels. Unlike many of the antibody receptors, CD23 is a C-type lectin. It is found on mature B cells, activated macrophages, eosinophils, follicular dendritic cells, and platelets. CD23 is found on B cells in B cell malignancies such as Hodgkin Lymphoma, Non-Hodgkin Lymphoma and B-cell chronic lymphocytic leukaemia.

CD30 is a cell membrane protein of the tumor necrosis factor receptor family and tumor marker. This receptor is expressed by activated, but not by resting, T and B cells. TRAF2 and TRAF5 can interact with this receptor, and mediate the signal transduction that leads to the activation of NF-kappaB. It is a positive regulator of apoptosis, and also has been shown to limit the proliferative potential of auto-reactive CD8 effector T cells and protect the body against autoimmunity. CD30 is found on T cell lymphomas including anaplastic large cell lymphoma as well as being expressed by B cell lymphomas including Hodgkin lymphoma.

CD43 is a major sialoglycoprotein on the surface of human T lymphocytes, monocytes, granulocytes, and some B lymphocytes, which appears to be important for immune function and may be part of a physiologic ligand-receptor complex involved in T-cell activation. CD43 is present in over 90% of T-cell lymphomas and may also be useful as part of a panel to demonstrate B-cell lymphoblastic lymphoma, since the malignant cells in this condition are often CD43 positive. It also stains granulocytes and their precursors, and therefore may be an effective marker for myeloid tumours,

CD44 is a cell-surface glycoprotein involved in cell–cell interactions, cell adhesion and migration. It is a receptor for hyaluronic acid and can also interact with other ligands, such as osteopontin, collagens, and matrix metalloproteinases (MMPs). It participates in a wide variety of cellular functions including lymphocyte activation, recirculation and homing, hematopoiesis, and tumor metastasis. CD44 is found in various splice formats and variations in CD44 are reported as cell surface markers for some breast and prostate cancer stem cells. It has also been seen as an indicator of increased survival time in epithelial ovarian cancer patients. CD44 variant isoforms are also relevant to the progression of head and neck squamous cell carcinoma.

CD47 is a membrane protein, which is involved in the increase in intracellular calcium concentration that occurs upon cell adhesion to extracellular matrix. The protein is also a receptor for the C-terminal cell binding domain of thrombospondin, and it may play a role in membrane transport and signal transduction. CD47 is a molecule found in many types of cancer and is used by bladder cancer cells to hide from normal scavenging by macrophages.

CD54 (also known as intracellular adhesion molecule 1) is a cell surface glycoprotein which is typically expressed on endothelial cells and cells of the immune system. It binds to integrins of type CD11a / CD18, or CD11b / CD18. CD54 is found on B-cell lymphoblastic lymphoma, mucosa associated lymphoid tissue lymphoma as well as other endothelial cancers as is implicated to play a role in metastasis.

CD55 (also known as complement decay accelerating factor) is a 70 kDa membrane protein that regulates the complement system on the cell surface. It prevents the assembly of the C3bBb complex (the C3-convertase of the alternative pathway) or accelerates the disassembly of preformed convertase, thus blocking the formation of the membrane attack complex. This glycoprotein is broadly distributed among hematopoietic and non-hematopoietic cells. It is found on many endothelial carcinomas including colorectal and prostate.

CD58 is a cell adhesion molecule expressed on Antigen Presenting Cells (APC), particularly macrophages. It binds to CD2 on T cells and is important in strengthening the adhesion between the T cells and Professional Antigen Presenting Cells. This adhesion occurs as part of the transitory initial encounters between T cells and Antigen Presenting Cells before T cell activation, when T cells are roaming the lymph nodes looking at the surface of APCs for peptide:MHC complexes. It is expressed on many lymphomas including B-cell lymphoblastic lymphoma and mucosa associated lymphoid tissue lymphoma.

CD59 inhibits the complement membrane attack complex by binding C5b678 and preventing C9 from binding and polymerizing. It is present on "self" cells to prevent complement from damaging them. It has a wide tissue distribution and has been implicated in breast and prostate cancers.

CD62L is a cell adhesion molecule found on lymphocytes. It belongs to the selectin family of proteins, which recognize sialylated carbohydrate groups. It is cleaved by ADAM17. It acts as a "homing receptor" for lymphocytes to enter secondary lymphoid tissues via high endothelial venules. It has been found on B cell lymphomas including chronic lymphocytic leukaemia as well as T cell lymphomas including adult T cell lymphoma.

CD95 (also known as Fas ligand) is a death receptor on the surface of cells that leads to programmed cell death (apoptosis). It is found on many cell types and has been implicated in many types of cancer including ovarian and colorectal carcinoma.

Accordingly, a first aspect of the invention provides an agent comprising:

(i) a T cell antigen, and

(ii) a binding partner for any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95,

wherein, following binding of the agent to a cell that expresses any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, the agent is internalised and the T cell antigen is presented on the surface of the cell in a form that can be recognised by a T cell.

For the avoidance of doubt, when the binding partner is for CD70, the agent will bind to a cell that expresses CD70, and so on.

T cell antigen

By a 'T cell antigen' we include the meaning of any antigen which can be presented to a T cell so as to elicit a T cell response. For example, the T cell antigen may be presented to a T cell by an MHC molecule or by a Group I CD1 molecule on the surface of the cell expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95. Once the antigen is presented on the surface of the cell, the cell is recognised as foreign and becomes the target of T cells, some of which have the natural function of eliminating foreign cells either infected by foreign organisms such as viruses, fungi, bacteria, mycobacteria or protozoa, or which have become cancerous (eg malignant). Thus, it will be appreciated that the T cell antigen may be one that is capable of being presented by a molecule on an unwanted cell expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95.

It will be appreciated that the T cell antigen is one that can elicit an existing T cell response in the subject to which the agent of the invention is administered. Typically, the T cell antigen is not one which generates a new primary T cell response for that antigen via cross-presentation in APCs. To put it another way, the T cell antigen is one to which a number of T cells in the subject are already sensitised to. Determining whether a subject's cells are sensitised to a given antigen can be done by contacting isolated peripheral mononuclear blood cells from the subject with the antigen and using standard assays for cell proliferation, as described further below and in the Examples.

In an embodiment, the agent of the invention is not one which generates a new T cell response specific for the T cell antigen contained in it. Accordingly, the invention includes an agent comprising a T cell antigen and a binding partner for any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, wherein, following binding of the agent to a cell that expresses any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, the agent is internalised and the T cell antigen is presented on the surface of the cell in a form that can be recognised by a T cell, and wherein the T cell antigen is capable of eliciting an existing T cell response in a subject.

By 'T cell', we include all types of T cell including CD4+, CD8+, γδ T cells, and NK-T cells. Preferably, the T cell is a CD4 + T cell, of which both helper and cytotoxic CD4 + T cells are known (Appay V (2004) *Clin Exp Immunol* **138(1)**: 10-13).

As is known in the art, the mechanism of antigen presentation will depend upon the type of T cell. It is understood that any presentation route may be used provided that the antigen elicits a T cell response. In other words, following internalisation of the agent of the invention, the T cell antigen must be capable of being presented on the surface of the cell such that it can elicit a T cell response. Preferably, the T cell antigen enters the MHC Class II processing machinery and is presented on an MHC Class II molecule, as described further below.

Preferably, the T cell antigen is an immunodominant antigen (eg an antigen that elicits an existing immunodominant response). By 'immunodominant' we include the meaning that the antigen elicits a T cell response with high magnitude, sensitivity, tissue homing characteristics and efficiency in killing antigen bearing cells. Generally, an immunodominant response comprises more than 0.1% of a subject's $CD8^+$ or $CD4^+$ T

cells. Determining the extent of a T cell response for a given antigen can be done for example by contacting isolated peripheral mononuclear blood cells from the subject with the antigen and using standard assays for cell proliferation known in the art. Suitable assays for determining the extent of an immune response include ELISpot, intracellular cytokine staining, HLA-peptide tetramer staining, proliferation assay, activation assays (eg CD69), CD107 mobilisation assays or metabolic assays (eg MTT).

Examples of suitable T cell antigens include any of a peptide, a polypeptide, a phosphopeptide or a lipid such as a phospholipid or a sphingolipid, and further examples of each of these are provided below. Most preferably, the T cell antigen is a peptide or polypeptide.

When the T cell antigen is a peptide or polypeptide, typically it is one that is capable of being recognised by a T cell receptor when bound to an MHC Class II molecule. The T cell antigen may be an MHC Class II restricted antigen that binds only to MHC Class II molecules. It is appreciated that the antigen may bind only to particular variant MHC Class II molecules (e.g. natural variants found in particular subjects), or that the antigen may be capable of binding to any MHC Class II molecule (i.e. the antigen is promiscuous).

In one embodiment, the T cell antigen is capable of binding to a MHC Class II molecule such as any of HLA-DP, HLA-DQ or HLA-DR. Common MHC Class II types include DR1, DR3, DR4, DR7, DR52, DQ1, DQ2, DQ4, DQ8 and DP1. MHC Class II molecules are expressed on immune cells including antigen presenting cells such as dendritic cells, B cells and macrophages. Thus, when the T cell antigen is MHC Class II restricted, the agent of the invention may be used to treat conditions such as lymphomas or autoimmune diseases. However, it will be appreciated that MHC Class II molecules can be upregulated on non-immune cells (eg upon stimulation with IFN-gamma) and so other conditions may also be treated.

An example of a promiscuous peptide that may be used is the PADRE MHC Class-II epitope defined in Alexander *et al* (2000) The Journal of Immunology 164: 1625-1633; aKXVAAWTLKAAaZC (a= d-Alanine, X = L-cyclohexylalanine, Z = aminocaproic acid)) (SEQ ID No: 1). Since this epitope is artificial, it would first need to be introduced to the patient in a vaccine to generate an immune response prior to administering the agent of the invention. Another promiscuous peptide that may be used is the tetanus fragment C peptide.

Conveniently, the T cell antigen is an immunogenic peptide that is recognised by an MHC Class II molecule. Such peptides usually have a length of 9 to 22 amino acids . Preferably, the peptide is an immunodominant peptide.

Examples of immunodominant peptides include viral derived peptides that elicit endogenous anti-viral responses. Thus, the peptide may be derived from an endogenous virus such as Varicella-Zoster virus, Herpes simplex virus, cytomegalovirus, Epstein Barr virus, or influenza. Particularly preferred examples, which may be used in combination with any of the binding partners described herein (eg CD22 binding partner) include peptides derived from human cytomegalovirus (CMV or Human herpesvirus 5/HHV5) or Epstein-Barr Virus (EBV or HHV4); herpesviruses such as HHV1, HHV2 and HHV3; influenza virus A; influenza virus B; rhinovirus; adenovirus; and Hepadnaviridae, specific examples of which are given below.

For human cytomegalovirus (HHV5) the immunodominant antigens are well characterised (see Sylwester AW *et al* J Exp Med. 2005 Sep 5;202(5):673-85, incorporated herein by reference), and so an antigen described in Sylwester *et al* may be used in the present invention. In particular, Sylester *et al* synthesised consecutive 15mer peptides, overlapping by 10 amino acids, for 213 predicted human CMV proteins. This generated 13,687 peptides that were arranged in ORF or sub-ORF specific mixes. Peptides derived from ORFs UL55 (gB), UL 83 (pp65), UL 86, UL 99 (pp28), UL 122 (IE2), UL 36, UL 48, UL32 (pp150), UL 113, IRS-1, UL 123 (IE1), UL25, UL 141, UL 52 and UL 82 (pp71) were found to elicit the most CD 4+ T cell responses, and so it is particularly preferred if the peptide is derived from one of these ORFs.

Particular cytomegalovirus T cell antigens that may be used are listed below.

CD4+ T cell epitopes for cytomegalovirus antigens such as pp65 include PQYSEHPTFTSQYRIQ (SEQ ID No: 1), FTSQYRIQGKLEYRHT (SEQ ID No: 2), LLQTGIHVRVSQPSL (SEQ ID No: 43), NPQPFMRPHERNGFT (SEQ ID No: 4), EPDVYYTSAFVFPTK (SEQ ID No: 5), IIKPGKISHIMLDVA (SEQ ID No: 6), AGILARNLVPMVATV (SEQ ID No: 7), KYQEFFWDANDIYRI (SEQ ID No: 8); for gB they include DYSNTHSTRYV (SEQ ID No: 9), CMLTITTARSKYPYH (SEQ ID No: 10), and VFETSGGLVVFWQGI (SEQ ID No: 11); for IE1 they include VRVDMVRHRIKEHMLKKYTQ (SEQ ID No: 12) and NYIVPEDKREMWMACIKELH (SEQ ID No: 13); and for gH they include HELLVLVKKAQL (SEQ ID No: 14).

For Epstein Barr Virus (EBV or HHV4), immunodominant proteins are also well characterised and are provided in Hislop AD *et al* Annu Rev Immunol. 2007;25:587-617 (incorporated herein by reference). A list of suitable T cell epitopes, adapted from Hislop *et al* is provided below.

Table 3: CD4+ T cell epitopes identified in EBV lytic and latent cycle proteins (adapted from Hislop *et al*)

| EBV Antigen | Epitope coordinates | Epitope sequence (SEQ ID No) | HLA restriction |
|---|---|---|---|
| *Latent cycle proteins* | | | |
| EBNA1 | 71-85 | RRPQKRPSCIGCKGT (15) | |
| | 403-417 | RPFFHPVGEADYFEY (16) | |
| | 429-448 | VPPGAIEQGPADDPGEGPST (17) | |
| | 434-458 | IEQGPTDDPGEGPSTGPRGQ GDGGR (18) | |
| | 455-469 | DGGRRKKGGWFGRHR (19) | |
| | 474-493 | SNPKFENIAEGLRVLLARSH (20) | |
| | 475-489 | NPKFENIAEGLRALL (21) | |
| | 479-498 | ENIAEGLRVLLARSHVERTT (22) | DQ7 |
| | 481-500 | IAEGLRALLARSHVERTTDE (23) | DQ2/3 |
| | 485-499 | LRALLARSHVERTTD (24) | |
| | 499-523 | EEGNWVAGVFVYGGSKTSLY NLRRG (25) | |
| | 509-528 | VYGGSKTSLYNLRRGTALAI (26) | DR11 |
| | 515-528 | TSLYNLRRGTALAI (27) | DR1 |
| | 518-530 | YNLRRGTALAIPQ (28) | DP3 |
| | 519-533 | NLRRGRTALAIPQCRL (29) | |
| | 519-543 | EEGNWVAGVFVYGGSKTSLYN LRRG (30) | |
| | 527-541 | AIPQCRLTPLSRLPF (31) | DR13 |
| | 529-543 | PQCRLTPLSRLPFGM (32) | DR14 |
| | 544-563 | APGPGPQPLRESIVCYFM (S43) (33) | |
| | 549-568 | PQPGPLRESIVCYFMVFLQT (S44) (34) | |
| | 551-570 | PGPLRESIVCYFMVFLQTHI (35) | DR1 |
| | 554-573 | LRESIVCYFMVFLQTHIFAE (36) | |
| | 554-578 | LRESIVCYFMVFLQTHIFAEVLKDA (37) | |
| | 561-573 | YFMVFLQTHIFAE (38) | DR11,12,13 |
| | 563-577 | MVFLQTHIFAEVLKD (39) | DR15 |

| | | | | |
|---|---|---|---|---|
| | | 564-583 | VFLQTHIFAEVLKDAIKDL (40) | DP5 |
| | | 574-593 | VLKDAIKDLVMTKPAPTCNI (41) | |
| | | 589-613 | PTCNIKVTVCSFDDGVDLPPW FPPM (42) | |
| | | 594-613 | RVTVCSFDDGVDLPPWFPPM (43) | |
| | | 607-619 | PPWFPPMVEGAAA (44) | DQ2 |
| | EBNA2 | 11-30 | GQTYHLIVDTLALHGGQTYH (45) | DR4 |
| | | 46-65 | IPLTIFVGENTGVPPPLPPP (46) | |
| | | 131-150 | MRMLWMANYIVRQSRGDRGL (47) | |
| | | 206-225 | LPPATLVPPRPTRPTTLPP (48) | |
| | | 276-295 | PRSPTVFYNIPPMPLPPSQL (49) | DR7,52a,52b,52c |
| | | 280-290 | TVFYNIPPMPL (50) | DQ2/DQ7 |
| | | 301-320 | PAQPPPGVINDQQLHHLPSG (51) | DR17 |
| | EBNA3A | 364-383 | EDLPCIVSRGGPKVKRPPIF (52) | DR15 |
| | | 780-799 | GPWVPEQWMFQGAPPSQGTP (53) | DR1 |
| | | 649-668 | QVADVVRAPGVPAMQPQYF (54) | |
| | EBNA3B | | | |
| | EBNA3C | 66-80 | NRGWMQRIRRRRRR (55) | |
| | | 100-119 | PHDITYPYTARNIRDAACRAV (56) | DR16 |
| | | 141-155 | ILCFVMAARQRLQDI (57) | DR13 |
| | | 386-400 | SDDELPYIDPNMEPV (58) | DQ5 |
| | | 401-415 | QQRPVMFVSRVPAKK (59) | |
| | | 546-560 | QKRAAPPTVSPSDTG (60) | |
| | | 586-600 | PPAAGPPAAGPRILA (61) | |
| | | 626-640 | PPVVRMFMRERQLPQ (62) | |
| | | 649-660 | PQCFWEMRAGREITQ (63) | |
| | | 741-760 | PAPQAPYQGYQEPPAPQAPY (64) | DR1/DR4 |
| | | 916-930 | PSMPFASDYSQGAFT (65) | |
| | | 961-986 | AQEILSDNSEISVFPK (66) | |
| | LMP1 | 11-30 | GPPRPPLGPPLSSSIGLALL (67) | DR7 & DR9 |
| | | 130-144 | LWRLGATIWQLLAFF (68) | |
| | | 181-206 | LIWMYYHGPRHTDEHHHDDS (69) | DR16 |
| | | 206-225 | QATDDSSHESDSNSNEGRHH (70) | DQ2 |
| | | 211-236 | SSHESDSNSNEGRHHLLVSG (71) | DQB1*0601 |
| | | 212-226 | SGHESDSNSNEGRHHH (72) | |
| | | 340-354 | TDGGGGHSHDSGHGG (73) | |
| | LMP2 | 73-87 | DYQPLGTQDQSLYLG (74) | DR4 or DR16 |
| | | 149-163 | STVVTATGLALSLLL (75) | |
| | | 169-182 | SSYAAAQRKLLTPV (76) | |

| | | | | |
|---|---|---|---|---|
| | | 189-208 | VTFFAICLTWRIEDPPFNSI (77) | DRB1*0901 |
| | | 194-213 | ICLTWRIEDPPFNSILFALL (78) | DRB1*1001 |
| | | 224-243 | VLVMLVLLILAYRRRWRRLT (79) | |
| | | 385-398 | STEFIPNLFCMLLL (80) | |
| | | 419-438 | TYGPVFMSLGGLLTMVAGAV (81) | DQB1*0601 |
| *Lytic Cycle Proteins* | | | | |
| | BHRF1 | 171-189 | AGLTLSLLVICSYLFISRG (82) | DR2 |
| | | 122-133 | PYYVVDLSVRGM (83) | DR4 |
| | | 45-57 | TVVLRYHVLLEEI (84) | DR4 |
| | BZLF1 | 174-188 | ELEIKRYKNRVASRK (85) | DR13 |
| | | 207-221 | KSSENDRLRLLLKQM (86) | DQB1*0402 |
| | BLLF1 | 61-81 | LDLFGQLTPHTKAVYQPRGA (87) | DRw15 |
| | (gp350) | 65-79 | FGQLTPHTKAVYQPR (88) | DRB1*1301 |
| | | 130-144 | VYFQDVFGTMWCHHA (89) | DQB1*0402 |
| | | 163-183 | DNCNSTNI TAVVRAQGLDVTL (90) | DRw11 |
| | BALF4 | 482-496 | AWCLEQKRQNMVLRE (91) | DPB1*1301 |
| | (gp110) | 575-589 | DNEIFLTKKMTEVCQ (92) | DRB1*0801 |

It is appreciated that the T cell antigen (e.g. peptide) may be one derived from a live vaccine such as Measles, Mumps, Rubella (MMR) or HHV3; or one derived from intracellular bacteria such as mycobacteria, particularly those evoked through immunization with BCG. Such peptides are well known in the art. Similarly, the T cell antigen (e.g. peptide) may be derived from the tetanus toxoid such as P2, P4 or P30. Thus, it will be understood that the T cell antigen (e.g. peptide) may be one that elicits an existing immune response in a subject that has been generated by prior vaccination against an infectious agent. It follows that in order to increase the number of T cells sensitised to a T cell antigen, it may be desirable to vaccinate or boost a subject with a vaccine that comprises the T cell antigen. For example, the subject may be vaccinated with a tetanus toxin, before being administered the agent of the invention comprising the relevant T cell antigen.

It will be appreciated that because many people are vaccinated in childhood with these vaccines, they are likely to contain T cells which are sensitized to these T cell antigens. Thus, in one embodiment the T cell antigen is one which is found in a childhood vaccine,

preferably one that is routinely used such as MMR, measles, BCG, yellow fever, polio, V2V and influenza.

Although not preferred, the T cell antigen (eg peptide) may also be one that elicits an existing immune response in a subject that has been generated by exposing that subject's T cells to the antigen *in vitro*.

Peptides can be produced by well known chemical procedures, such as solution or solid-phase synthesis, or semi-synthesis in solution beginning with protein fragments coupled through conventional solution methods as is known in the art. Alternatively, the peptide can be synthesised by established methods including recombinant methods.

Although it is preferred that the T cell antigen is a polypeptide or peptide, it is known that other antigens are also capable of eliciting immune responses and so have utility in the present invention. For example, γδ T cells do not recognise MHC-associated peptide antigens and are not MHC restricted. Some γδ T cell clones recognise small phosphorylated molecules, pyrophosphorylated compounds (eg HMBPP (E-4-hydroxy-3-methyl-but-2-enyl-pyrophosphate) and IPP (isopentenyl pyrophosphate)), alkyl amines or lipids (e.g. phosphorylated lipids) that may be presented by 'non-classical' class I MHC-like molecules called CD1 molecules. Similarly, NK-T cells (e.g. Vα24Vβ11 cells) recognise lipids (e.g. ceramides such as α-gal-ceramide) bound to CD1 molecules. Thus, the T cell antigen may be any of these molecules that are known to elicit a T cell response. Of course, the T cell antigen must be one that is capable of being presented on any of these molecules following internalisation of the agent into the cell.

When the agent is used as described below to treat autoimmune or allergic diseases, it will be appreciated that the T cell antigen may be an autoantigen or allergen respectively. In this way the immune response that is contributing to the disorder is redirected to unwanted cells so as to combat the disorder.

It is appreciated that the T cell antigen may be chemically modified provided that it is still capable of eliciting a T cell response. Such chemical modification may include, for instance, the addition of a metal such as nickel, since it has been shown that in certain allergic patients there are T cells which recognise a peptide with a bound nickel atom (Romagnoli *et al* 1991, EMBO J 10: 1303-1306). The T cell antigen can also be modified by an organic molecule which enhances the immunogenicity (Romero *et al* 1993,

J Immunol 150: 3825-3831). Other modifications include phosphorylation, acetylation, alkylation, acylation, amidation, glycosylation, methylation, citrulination, nitration, sulphation and hydroxylation, forming salts with acids or bases, forming an ester or amide of a terminal carboxyl group, and attaching amino acid protecting groups such as N-t-butoxycarbonal.

When the T cell antigen is a peptide, it is appreciated that it may comprise naturally occurring amino acids encoded by DNA, and/or one or more non-natural amino acids, including amino acids in the "D" isomeric form or incorporating the use of N-methylated amino acids or beta amino acids or peptoids, provided that it is recognised by the corresponding T cell. Thus, the peptide may be a peptide 'mimetic' ie peptidomimetic which mimics the structural features of any of the peptides mentioned above. For example, the T cell antigen may be a retro-inverso peptide.

Similarly, the T cell antigen, when a peptide, may be a mimotope, ie a peptide composed of natural or non-natural amino acids that mimics the structure of the natural epitope. Mimotopes often stimulate T cells more potently.

Preferably, the T cell antigens are substantially non-toxic in the absence of T lymphocytes. By 'substantially non-toxic' we mean that the antigens have considerably lower or preferably no detectable toxicity, compared to toxins such as Pseudomonas exotoxin.

The skilled person will be able to identify further T cell antigens that may be used in the invention using the database available at http://www.immuneepitope.org (Vita R, Zarebski L, Greenbaum JA, Emami H, Hoof I, Salimi N, Damle R, Sette A, Peters B. The immune epitope database 2.0. *Nucleic Acids Res.* 2010 Jan; 38(Database issue):D854-62. Epub 2009 Nov 11).

Binding partner

By 'binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95', we include the meaning of any molecule that binds to any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 respectively. In this way, the agent of the invention can bind to the surface of cells that express any of CD70, CD74, CD22,

HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95.

By 'CD70' we include human CD70, the amino sequence of which is provided in Figure 1A and which has Accession Number P32970. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD70 is not limited to the binding partner of human CD70 having the sequence listed in Figure 1A, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD70 also includes binding partners of CD70 in other species which have an orthologous sequence to that in Figure 1A, for example CD70 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD74' we include human CD74, the amino sequence of which is provided in Figure 1B and which has Accession Number P04233. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD74 is not limited to the binding partner of human CD74 having the sequence listed in Figure 1B, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD74 also includes binding partners of CD74 in other species which have an orthologous sequence to that in Figure 1B, for example CD74 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD22' we include human CD22, the amino sequence of which is provided in Figure 1B and which has Accession Number P20273. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD22 is not limited to the binding partner of human CD22 having the sequence listed in Figure 1C, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD22 also includes binding partners of CD22 in other species which have an orthologous sequence to that in Figure 1C, for example CD22 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'HLA-DR' we include human HLA-DR, the amino sequence of which is provided in Figure 1D and which has Accession Number Q29769. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of HLA-DR is not limited to the binding partner of human HLA-DR having the sequence listed in Figure 1D, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of HLA-DR also includes binding partners of HLA-DR in other species which have an orthologous sequence to that in Figure 1D, for example HLA-DR from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD23' we include human CD23, the amino sequence of which is provided in Figure 1E and which has Accession Number P06734. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD23 is not limited to the binding partner of human CD23 having the sequence listed in Figure 1E, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD23 also includes binding partners of CD23 in other species which have an orthologous sequence to that in Figure 1E, for example CD23 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD30' we include human CD30, the amino sequence of which is provided in Figure 1F and which has Accession Number P28908. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD30 is not limited to the binding partner of human CD30 having the sequence listed in Figure 1F, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD30 also includes binding partners of CD30 in other species which have an orthologous sequence to that in Figure 1F, for example CD30 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD43' we include human CD43, the amino sequence of which is provided in Figure 1G and which has Accession Number P16150. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD43 is not

limited to the binding partner of human CD43 having the sequence listed in Figure 1G, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD43 also includes binding partners of CD43 in other species which have an orthologous sequence to that in Figure 1G, for example CD43 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD44' we include human CD44, the amino sequence of which is provided in Figure 1H and which has Accession Number P16070. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD44 is not limited to the binding partner of human CD44 having the sequence listed in Figure 1H, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD44 also includes binding partners of CD44 in other species which have an orthologous sequence to that in Figure 1H, for example CD44 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD47' we include human CD47, the amino sequence of which is provided in Figure 1I and which has Accession Number P08722. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD47 is not limited to the binding partner of human CD47 having the sequence listed in Figure 1I, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD47 also includes binding partners of CD47 in other species which have an orthologous sequence to that in Figure 1I, for example CD47 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD54' we include human CD54, the amino sequence of which is provided in Figure 1J and which has Accession Number P05362. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD54 is not limited to the binding partner of human CD54 having the sequence listed in Figure 1J, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD54 also includes binding partners of CD54 in other species which have an

orthologous sequence to that in Figure 1J, for example CD54 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD55' we include human CD55, the amino sequence of which is provided in Figure 1K and which has Accession Number P08174. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD55 is not limited to the binding partner of human CD55 having the sequence listed in Figure 1K, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD55 also includes binding partners of CD55 in other species which have an orthologous sequence to that in Figure 1K, for example CD55 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD58' we include human CD58, the amino sequence of which is provided in Figure 1L and which has Accession Number P19256. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD58 is not limited to the binding partner of human CD58 having the sequence listed in Figure 1L, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD58 also includes binding partners of CD58 in other species which have an orthologous sequence to that in Figure 1L, for example CD58 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD59' we include human CD59, the amino sequence of which is provided in Figure 1M and which has Accession Number P13987. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD59 is not limited to the binding partner of human CD59 having the sequence listed in Figure 1M, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD59 also includes binding partners of CD59 in other species which have an orthologous sequence to that in Figure 1M, for example CD59 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD62L' we include human CD62L, the amino sequence of which is provided in Figure 1N and which has Accession Number P14151. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD62L is not limited to the binding partner of human CD62L having the sequence listed in Figure 1N, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD62L also includes binding partners of CD62L in other species which have an orthologous sequence to that in Figure 1N, for example CD62L from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

By 'CD95' we include human CD95, the amino sequence of which is provided in Figure 1O and which has Accession Number P25445. However, it is well known that certain polypeptides are polymorphic, and it is appreciated that some natural variation of this sequence may occur. Thus, in an embodiment, the binding partner of CD95 is not limited to the binding partner of human CD95 having the sequence listed in Figure 1O, but includes binding partners to naturally occurring variants thereof in which one or more of the amino acid residues have been replaced with another amino acid. The binding partner of CD95 also includes binding partners of CD95 in other species which have an orthologous sequence to that in Figure 1O, for example CD95 from other mammals such as horse, dog, pig, cow, sheep, rat, mouse, guinea pig or a primate.

As described further below, the agents of the invention may be administered to subjects for use in medicine. With respect to the subject to which the agent is administered, it is preferred that the agent comprises a binding partner that binds to any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 of that species. For example, when the subject is a human, the agent comprises a binding partner of human any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, and so on.

Preferably, the binding partner binds selectively to any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95. For example, it is preferred if the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 has a $K_d$ value (dissociation constant) which is at least five or ten times lower (i.e. higher affinity) than for at least one other entity expressed by that cell, and preferably more than 100 or 500 times lower. More preferably, the binding partner of any of CD70, CD74, CD22,

HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 has a $K_d$ value more than 1000 or 5000 times lower than for at least one other entity expressed by that cell. $K_d$ values can be determined readily using methods well known in the art.

The binding partner may be any of a polypeptide, a peptide, a small molecule or a peptidomimetic.

In a preferred embodiment, the binding partner is an antibody that binds to CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95.

The binding partner may be an anti-CD70 antibody such as any of BU69 or SGN-70 or SGN-75 or a commercial antibody from eBioscience (anti-human CD74 purified, clone LN2) (Epstein *et al*, 1984, *Immunol.* **133(2)**: 1028; Lamb *et al*, 1991, *PNAS* **88(14)**: 5998).

The binding partner may be an anti-CD74 antibody such as milatuzumab (Becker-Herman *et al*, 2005, *Mol. Biol. Cell.* **16(11)**: 5061).

The binding partner may be an anti-CD22 antibody such as Epratuzumab (Stein R. *et al*, *Cancer Immunol Immunother* **37**: 293-298 (October 1993).

The binding partner may be an anti-HLA-DR antibody such as a commercial antibody from eBioscience (anti-human HLA-DR, clone L243) (Brodsky FM. A. *Immunogenetics* 1984;**19(3)**: 179-94; Engleman EG, Warnke R, Fox RI, Dilley J, Benike CJ, Levy R. *Proc Natl Acad Sci USA* 1981 Mar; **78(3)**: 1791-5.).

The binding partner may be an anti-CD23 antibody such as a commercial antibody from eBioscience (anti-human CD23 purified, clone EBVCS2) (Knapp, W., B. Dorken, *et al* eds. (1989) Leucocyte Typing IV: White Cell Differentiation Antigens. Oxford University Press. New York; McMichael, A.J., P.C.L. Beverly, *et al* eds. (1987) Leucocyte Typing III: White Cell Differentiation Antigens. Oxford University Press. New York; Bernard, A., *et al* eds. (1981) Leukocyte Typing. Springer-Verlag.

The binding partner may be an anti-CD30 antibody such as a commercial antibody from eBioscience (anti-human CD30 purified, clone BerH2) Tamiolakis D, *et al Int J Biol Sci*

2005; **1**: 135-140; Polski JM, Janney CG. *Ber-H2 Mod Pathol.* 1999 Sep; 12(9): 903-6; Horie R, Watanabe, T. *J Immunol* 1998; **10**: 457-470.)

The binding partner may be an anti-CD43 antibody such as a commercial antibody from eBioscience (anti-human CD43 purified, clone eBio84-3C1) (Borche L, Lozano F, Vilella R, Vives J. *Eur J Immunol.* 1987 Oct; **17(10)**:1523-6; Schlossman, S., L. Bloumsell, *et al* eds. 1995. Leucocyte Typing V: White Cell Differentiation Antigens. Oxford University Press. New York.).

The binding partner may be an anti-CD44 antibody such as a commercial antibody from eBioscience (anti-human CD44 purified, clone IM7) (Trowbridge, I. S., J. Lesley, *et al* 1982. *Immunogenetics* **15(3)**: 299-312; Lesley, J. and I. S. Trowbridge 1982. *Immunogenetics* **15(3)**: 313-20; Maiti A, Maki G, Johnson P. *Science.* 1998. Oct 30; **282(5390)**: 941-3.).

The binding partner may be an anti-CD47 antibody such as a commercial antibody from eBioscience (anti-human CD47 purified, clone B6H12) (Grimbert P, Bouguermouh S, *et al J Immunol.* 2006 Sep 15; **177(6)**: 3534-41; Lagadec P, Dejoux O, *et al* 2003 Jun 15; **101(12)**: 4836-43.).

The binding partner may be an anti-CD54 antibody such as a commercial antibody from eBioscience (anti-human CD54 purified, clone eBio KAT1) (Lehmann JC, *et al J Immunol.* 2003 Sep 1; **171(5)**: 2588-93; Arai K, *et al Int J Pancreatol.* 1999 Aug; **26(1)**: 23-31).

The binding partner may be an anti-CD55 antibody such as a commercial antibody from eBioscience (anti-human CD55 purified, clone 143-30) (Knapp, W., B. Dorken, *et al* eds. (1989). Leucocyte Typing IV: White Cell Differentiation Antigens. Oxford University Press. New York).

The binding partner may be an anti-CD58 antibody such as a commercial antibody from eBioscience (anti-human CD58 purified, clone TS2/9) (Ariel O, *et al, Cellular Signaling* 2009: **21**: 1100-1108; Osborn L, *et al J. Exp. Med.* January 1995; **181**: 429-434.).

The binding partner may be an anti-CD59 antibody such as a commercial antibody from eBioscience (anti-human CD59 purified, clone OV9A2) (Alegretti AP *et al Cell Immunol.* 2010; **265(2)**: 127-32; Deckert M, *et al Eur J Immunol.* 1992 Nov; **22(11)**: 2943-7).

The binding partner may be an anti-CD62L antibody such as a commercial antibody from eBioscience (anti-human CD59 purified, clone DREG-56) (Jutila MA, *et al J. Immunol*, Aug 15;169(4): 1768-73; Schlossman, S., L. Bloumsell *et al* eds. 1995. Leucocyte Typing V: White Cell Differentiation Antigens. Oxford University Press. New York).

The binding partner may be an anti-CD95 antibody such as a commercial antibody from eBioscience (anti-human CD59 purified, clone APO-1-1) (Rajasagi, M *et al Journal of Leukocyte Biology* 2009; **85**: 251-261; Fluhr, H *et al Journal of Cell Science* 2007; **120**: 4126-4133.).

Alternatively, the binding partner may be any molecule or part thereof that specifically binds, in a non-immune sense, to any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95. Thus, the specific binding partner may be any of a hormone, a growth factor, a cytokine, or a receptor ligand (e.g. agonist or antagonist).

CD70 is known to bind to CD27, and so in one embodiment, the binding partner of CD70 is CD27.

CD74 is known to bind to macrophage migration inhibitory factor (MIF), and so in one embodiment, the binding partner of CD74 is MIF.

CD23 is known to bind to IgE, and so in one embodiment, the binding partner of CD23 is IgE.

CD30 is known to bind to CD30L, and so in one embodiment, the binding partner of CD30 is CD30L.

CD43 is known to bind to sialic acid residues, and so in one embodiment, the binding partner of CD43 is sialic acid.

CD44 is known to bind to hyaluronic acid, collagen and oestopontin, and so in one embodiment, the binding partner of CD44 is any of hyaluronic acid, collagen and oestopontin.

CD47 is known to bind to thrombospondin, and so in one embodiment, the binding partner of CD47 is thrombospondin.

CD54 is known to bind to LFA-2, and so in one embodiment, the binding partner of CD54 is LFA-2.

CD58 is known to bind to CD2, and so in one embodiment, the binding partner of CD58 is CD2.

CD62L is known to bind to peripheral lymphonode addressin, and so in one embodiment, the binding partner of CD62L is peripheral lymphonode addressin.

CD95 is known to bind to FasL (CD95L), and so in one embodiment, the binding partner of CD95 is FasL (CD95L).

HLA-DR is known to bind to CD4, and so in one embodiment, the binding partner of HLA-DR is CD4.

As used herein, the term "antibody" includes but is not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, fragments produced by a Fab expression library and bispecific antibodies. Such fragments include fragments of whole antibodies which retain their binding activity for a target substance, Fv, F(ab') and F(ab')2 fragments, as well as single chain antibodies (scFv), fusion proteins and other synthetic proteins which comprise the antigen-binding site of the antibody. A binding partner comprising only part of an antibody may be advantageous by virtue of optimising the rate of clearance from the blood and may be less likely to undergo non-specific binding due to the Fc part. Also included are domain antibodies (dAbs), diabodies, camelid antibodies and engineered camelid antibodies. Furthermore, for administration to humans, the antibodies and fragments thereof may be humanised antibodies, which are now well known in the art (Janeway *et al* (2001) *Immunobiology.*, 5th ed., Garland Publishing); An *et al* (2009) *Therapeutic Monoclonal Antibodies*: From Bench to Clinic, ISBN: 978-0-470-11791-0).

Also included are asymmetric IgG-like antibodies (eg triomab/quadroma, Trion Pharma/Fresenius Biotech; knobs-into-holes, Genentech; Cross MAbs, Roche; electrostatically matched antibodies, AMGEN; LUZ-Y, Genentech; strand exchange engineered domain (SEED) body, EMD Serono; biolonic, Merus; and Fab-exchanged antibodies, Genmab), symmetric IgG-like antibodies (eg dual targeting (DT)-Ig,

GSK/Domantis; two-in-one antibody, Genentech; crosslinked MAbs, karmanos cancer center; $mAb^2$, F-star; and Cov X-body, Cov X/Pfizer), IgG fusions (eg dual variable domain (DVD)-Ig, Abbott; IgG-like bispecific antibodies, Eli Lilly; Ts2Ab, Medimmune/AZ; BsAb, ZymoGenetics; HERCULES, Biogen Idec; TvAb, Roche) Fc fusions (eg ScFv/Fc fusions, Academic Institution; SCORPION, Emergent BioSolutions/Trubion, ZymoGenetics/BMS; dual affinity retargeting technology (Fc-DART), MacroGenics; dual $(ScFv)_2$-Fab, National Research Center for Antibody Medicine) Fab fusions (eg $F(ab)_2$, Medarex/AMGEN; dual-action or Bis-Fab, Genentech; Dock-and-Lock (DNL), ImmunoMedics; bivalent bispecific, Biotechnol; and Fab-Fv, UCB-Celltech), ScFv- and diabody-based antibodies (eg bispecific T cell engagers (BiTEs), Micromet; tandem diabodies (Tandab), Affimed; DARTs, MacroGenics; Single-chain diabody, Academic; TCR-like antibodies, AIT, Receptor Logics; human serum albumin ScFv fusion, Merrimack; and COMBODIES, Epigen Biotech), IgG/non-IgG fusions (eg immunocytokins, EMDSerono, Philogen, ImmunGene, ImmunoMedics; superantigen fusion protein, Active Biotech; and immune mobilising mTCR Against Cancer, ImmTAC) and oligoclonal antibodies (eg Symphogen and Merus).

The antibody may possess any of the antibody-like scaffolds described by Carter (2006) "Potent antibody therapeutics by design", *Nat Rev Immunol.* **6(5)**: 343-57, and Carter (2011) "Introduction to current and future protein therapeutics: a protein engineering perspective", *Exp Cell Res.* **317(9)**: 1261-9. incorporated herein by reference, together with the specificity determining regions described herein. Thus, the term "antibody" also includes affibodies and non-immunoglobulin based frameworks. Examples include adnectins, anticalins, affilins, trans-bodies, darpins, trimerX, microproteins, fynomers, avimers, centgrins and kalbitor (ecallantide).

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 convenient production in the laboratory and economical production on a commercial scale. Also, such fragments allow for increased toxological safety because of the lack of the Fc component.

The antibody may be of any of the IgG, IgE, IgA, IgM and IgD classes and may be derived from any species. If the antibody is an IgG, it may be any of IgG1, IgG2, IgG3 or IgG4. It is preferred, however, that when the agent is for administration to a particular

host, that the antibody, or at least the constant regions thereof, are derived from that host. For example, when the agent is to be administered to a human, the antibody is preferably a human antibody or a humanized antibody, and so on.

Suitable antibodies that bind to CD70 or CD74 can be made by the skilled person using technology long-established in the art. Methods of preparation of monoclonal antibodies and antibody fragments are well known in the art and include hybridoma technology (Kohler & Milstein (1975) "Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**: 495–497); antibody phage display (Winter *et al* (1994) "Making antibodies by phage display technology." *Annu. Rev. Immunol.* **12:** 433–455); ribosome display (Schaffitzel *et al* (1999) "Ribosome display: an *in vitro* method for selection and evolution of antibodies from libraries." *J. Immunol. Methods* **231**: 119–135); and iterative colony filter screening (Giovannoni *et al* (2001) "Isolation of anti-angiogenesis antibodies from a large combinatorial repertoire by colony filter screening." *Nucleic Acids Res.* **29:** E27). Further, antibodies and antibody fragments suitable for use in the present invention are described, for example, in the following publications: "*Monoclonal Hybridoma Antibodies: Techniques and Application*", Hurrell (CRC Press, 1982); "*Monoclonal Antibodies: A Manual of Techniques*", H. Zola, CRC Press, 1987, ISBN: 0-84936-476-0; "*Antibodies: A Laboratory Manual*" 1st Edition, Harlow & Lane, Eds, Cold Spring Harbor Laboratory Press, New York, 1988. ISBN 0-87969-314-2; "*Using Antibodies: A Laboratory Manual*" 2nd Edition, Harlow & Lane, Eds, Cold Spring Harbor Laboratory Press, New York, 1999. ISBN 0-87969-543-9; and "*Handbook of Therapeutic Antibodies*" Stefan Dübel, Ed., 1st Edition, - Wiley-VCH, Weinheim, 2007. ISBN: 3-527-31453-9.

Internalisation and presentation on surface of cell

By "the agent is internalised and the T cell antigen is presented on the surface of the cell in a form that can be recognised by a T cell", we include the meaning that the agent is taken into the cell (e.g. by endocytosis) and the T cell antigen is subsequently presented on the surface of the cell in a form that allows recognition by a T cell. Such recognition can be readily determined by assessing activation of the T cell, for example after contacting the T cell with the cell presenting the T cell antigen and using standard assays for cell proliferation known in the art. Suitable assays for determining the extent of an immune response include ELISpot, intracellular cytokine staining, proliferation assay, activation assays (eg CD69), CD107 mobilisation assays or metabolic assays (eg MTT).

Also suitable are assays to detect activation-induced secreted cytokines, for example using ELISA or multiplexed bead technologies.

Internalisation can be assessed using any suitable assay known in the art such as a flow cytometric based assay. For example the agent may be coupled to a fluorochrome such as fluorescein isothiocyanater (FITC) and target cells labelled with the agent. After 1-24 hours, a weak acid (eg citric acid) is added to the target cells and after washing the cells would be analysed on a flow cytometer. The acid will quench the fluorochrome if it is still on the cell surface and there would be no signal on the flow cytometer, thereby demonstrating that the agent has not been internalised. If there is internalisation of the agent, the fluorochrome is not accessible to the acid and there would be no quenching effect. This would mean that there would be a positive signal on the flow cytometer, thereby demonstrating internalisation of the agent.

It will be appreciated that one can determine whether the T cell antigen is being presented to a T cell following internalisation of the T cell antigen rather than by external loading of the antigen onto a cell's surface, using routine procedures. For example, cells may be exposed to the agent of the invention under conditions in which internalisation is prevented. Suitable conditions may be lightly fixing the cells using agents such as paraformaldehyde or glutaraldehyde, or performing the experiments at temperatures around 4°C or below. If internalisation is a requisite to the T cell antigen being presented, there should be no activation of T cells once internalisation is suppressed. Alternatively, one may use inhibitors of intracellular processing pathways to establish whether presentation of the T cell antigen follows internalisation or is the result of external loading. For example, inhibitors of the MHC Class II intracellular processing pathway may be used as is well known in the art and described further below.

In a preferred embodiment, the T cell antigen is one that is internalised and enters the classical MHC Class II processing pathway. For example, the T cell antigen peptide may be released from the agent by proteolytic degradation in endocytic vesicles and become bound to MHC Class II molecules before being exported to the cell surface. Assessing whether a T cell antigen is processed by the MHC Class II pathway is standard practice in art, and may include testing for processing in the presence and absence of known inhibitors of the pathway such as chloroquinone and monensin.

To facilitate processing of the T cell antigen inside the cell, it is preferred if the T cell antigen is attached to the binding partner for any of CD70, CD74, CD22, HLA-DR, CD23,

CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 such that the T cell antigen can be released from the binding partner within the cell that expresses any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95.Suitable attachments are provided below and include various heterobifunctional crosslinkers such as sulfo-SMCC which can attach peptides to free amine groups, eg on the external surface of an antibody. Other crosslinkers may be used to attach peptides to other functional groups (eg carboxyl, hydroxyl moieties) or to carbohydrate groups. Typically, the attachments are covalent, although strong non-covalent attachments such as Biotin-Avidin or hapten-specific antibodies (eg Digoxigenin) may be used.

In an embodiment, the T cell antigen can be released from the binding partner by an intracellular protease.

Without wishing to be bound by any theory, the inventors believe that, following internalisation of an agent comprising an MHC Class II restricted peptide into the cell, the agent will be processed in the same manner as the MHC Class II processing pathway where the endolysosome would become acidified. Acidification would activate various endosomal and lysosomal based proteases, such as cathepsins, which would together break down the agent and thereby release the peptide from the agent. It will be appreciated that the proteolysis is not acting on the peptide itself. The peptide may then be loaded onto MHC Class II molecules and presented on the cell surface.

Release of the T cell antigen from the binding partner may be tested using T cell antigen specific T cells that would recognise the T cell antigen presented on the cell surface. If the T cell antigen was released, T cells would recognise the T cell antigen on the cell surface and this would be determined by a positive signal in T cell recognition assays as mentioned previously. If the T cell antigen was not released, there would be a negative signal in T cell recognition assays. The T cell antigen may also be labelled using a fluorophore and techniques such as direct cellular imaging used to assess distribution of the T cell antigen.

It will be appreciated that the T cell antigen is not attached to the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 in such a way that the T cell antigen can be released from the binding partner extracellularly. Rather, the T cell antigen must be internalised into the cell expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47,

CD54, CD55, CD58, CD59, CD62L or CD95 and presented on its surface. Preferably, therefore, the T cell antigen is not attached to the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 in a way that the T cell antigen can be released from the binding partner extracellularly, for example by any one or more of an extracellular protease, a nuclease, a lipase, a lyase, a phosphatase or a carbohydrase. To ensure this, it is preferred if the agent does not include a site cleavable by an extracellular molecule (e.g. protease, nuclease, lipase, lyase, phosphatise, carbohydrase) that would act to release the T cell antigen from the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95. For example, the agent may not include a site (eg a specific protease cleavage site) cleavable by an extracellular molecule (eg a specific protease) residing in the vicinity of a cell expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, such as a cancer cell. It will be appreciated that this will reduce the necessary size of the agent of the invention. For example, where the T cell antigen is a peptide, the agent of the invention typically comprises (i) a peptide having a length less than 22 amino acids (e.g. less than 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 amino acids) which peptide comprises or consists of the T cell antigen, and (ii) a binding partner (e.g. antibody) that is attached to the peptide.

Determining whether a given sequence can be cleaved by a protease, and if so which protease, is routine practice for the skilled person. There has been a lot of research into proteolytic cleavage sequences, with many programs available to determine the proteolytic activity towards a given sequence (eg Sigma Aldrich programs). There are also databases (eg MEROPs and PMAP) that contain a wealth of information about proteolysis and protease recognition sequences. Any suitable method may be used.

Synthesis of agent of invention

Conveniently, the T cell antigen is joined to the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 by a linker. By 'linker' we include the meaning of a chemical moiety that attaches the binding partner to the T cell antigen.

In an embodiment, the linker does not include a site cleavable by an extracellular molecule, such as an extracellular protease. Thus, in an embodiment any moiety that

joins the binding partner to the T cell antigen does not include a site cleavable by an extracellular molecule such as an extracellular protease.

It is appreciated that the T cell antigen may either be bound covalently or non-covalently to the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95. Preferably, the T cell antigen is covalently attached to the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95.

In one embodiment, the T cell antigen and binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, are covalently attached by a linker.

Thus, the T cell antigen (e.g. peptide) and binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, may be conveniently linked by any of the conventional ways of cross-linking molecules, such as those generally described in O'Sullivan *et al Anal. Biochem.* (1979) 100, 100-108. For example, one of the T cell antigen (e.g. peptide) or binding partner of CD70 or CD74 may be enriched with thiol groups and the other 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), a heterobifunctional cross-linking agent which incorporates a disulphide bridge between the conjugated species. Amide and thioether bonds, for example achieved with m-maleimidobenzoyl-N-hydroxysuccinimide ester, are generally more stable *in vivo* than disulphide bonds.

It is known that bis-maleimide reagents allow the attachment of a thiol group (e.g. thiol group of a cysteine residue of an antibody) to another thiol-containing moiety (e.g. thiol group of a T cell antigen or a linker intermediate), in a sequential or concurrent fashion. Other functional groups besides maleimide, which are reactive with a thiol group include iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate.

Further useful cross-linking agents include S-acetylthioglycolic acid N-hydroxysuccinimide ester (SATA) which is a thiolating reagent for primary amines which allows deprotection of the sulphydryl group under mild conditions (Julian *et al* (1983) *Anal. Biochem.* **132**, 68), dimethylsuberimidate dihydrochloride and N,N'-o-phenylenedimaleimide.

Particularly preferred crosslinking agents include sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (Sulfo-SMCC), sulfosuccinimidyl 6-(3'-[2-pyridyldithio]-propionamido) hexanoate (Sulfo-LC-SPDP) and *N*-[ß-Maleimidopropionic acid] hydrazide, trifluoroacetic acid salt (BMPH).

It will be understood that a large number of homobifunctional and heterobifunctional crosslinking chemistries would be appropriate to join the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 to the T cell antigen, and any such chemistry may be used. For example, Click Chemistry using Staudinger Ligation Chemistry (phosphine-azido chemistry) may be used.

It is appreciated that the T cell antigen and binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 do not need to be cross-linked directly to each other, but may be attached via one or more spacer moieties. For example, the T cell antigen may be crosslinked to a chemical moiety which in turn is crosslinked to the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95. Generally, a spacer moiety may serve to prevent steric hindrance; however, since the agent is expected to be broken down intracellularly such that the T cell antigen is released, it will be understood that one or more spacer moieties are not required.

In a specific embodiment where the T cell antigen and binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 are covalently attached and where both the antigen and binding partner are peptides or polypeptides, it is appreciated that the two components may be part of a fusion polypeptide that may be encoded by a nucleic acid molecule. The invention includes such a nucleic acid molecule and host cells containing them. For example, an antibody binding partner may be genetically engineered to contain the T cell antigen using genetic engineering techniques well established in the art. Thus, it will be appreciated that the T cell antigen may be embedded within, or at the termini of, the polypeptide sequence of the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, provided that it can be released so as to be capable of being presented on the surface of a cell that expresses any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 following internalisation. Suitably,

the T cell antigen and the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 are joined so that both portions retain their respective activities such that the agent may be targeted to a cell expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 and the T cell antigen may be presented by the cell so as to elicit an immune response. The T cell antigen and binding partner may be joined by a linker peptide. Suitable linker peptides are those that typically adopt a random coil conformation, for example the polypeptide may contain alanine or proline or a mixture of alanine plus proline residues. Preferably, the linker contains between 2 and 100 amino acid residues, more preferably between 2 and 50 and still more preferably between 4 and 20. However, as discussed above, it will be realised that a linker peptide is not essential given that the agent is broken down intracellularly such that the T cell antigen is released.

Polynucleotides which encode suitable binding partners of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 are known in the art or can be readily designed from known sequences such as from sequences of proteins known to interact with any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 or contained in nucleotide sequence databases such as the GenBank, EMBL and dbEST databases. Polynucleotides which encode suitable T cell antigens are known in the art or can readily be designed from known sequences and made.

Polynucleotides which encode suitable linker peptides can readily be designed from linker peptide sequences and made.

Thus, polynucleotides which encode the agents used in the invention can readily be constructed using well known genetic engineering techniques.

The nucleic acid is then expressed in a suitable host to produce an agent of the invention. Thus, the nucleic acid encoding the agent of the invention may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the agent of the invention of the invention.

It is appreciated that the nucleic acid encoding the agent of the invention may be joined

to a wide variety of other nucleic acid sequences for introduction into an appropriate host. The companion nucleic acid will depend upon the nature of the host, the manner of the introduction of the nucleic acid into the host, and whether episomal maintenance or integration is desired, as is well known in the art.

In an alternative embodiment, the T cell antigen and the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 are non-covalently attached. However, it will be appreciated that the non-covalent attachment must be sufficiently stable to allow the agent to be localised to the cell following administration of the agent to a subject, and to allow the T cell antigen to be presented on its surface. Typically, non-covalent bindings should have an affinity with a $K_d < 10^{-9}$. For non-covalent bindings, immunological bindings or such binding as via biotin/avidin or streptavidin, respectively, are preferred. For example, the binding partner of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 may be a bispecific antibody, one specificity of which is directed to an entity expressed by the unwanted cell and one specificity of which is directed to the T cell antigen or part thereof. Also, it is possible to couple the T cell antigen to another substance against which, in turn, the specificity of the bispecific antibody will be directed to. For instance, the T cell antigen may contain further peptidic sequences which are recognised by the bispecific antibody. Another possibility involves coupling the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, for example to streptavidin whilst the T cell antigen is coupled to biotin, and vice versa. Other means by which non-covalent interactions can be formed include leucine zipper sequences or affinity bonds. In any event, the attachment between the T cell antigen and the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 must be such that, following internalisation of the agent into the cell expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 respectively, the T cell antigen can be presented on the surface of the cell in a form that can be recognised by a T cell.

Amino acid residues described herein are generally in the natural "L" isomeric form. However, residues in the "D" isomeric form can be substituted for L-amino acid residues in certain situations, provided that the T cell antigen of the agent can still be presented on the surface of the cell expressing CD70 or CD74 in a form that can be recognised by a T cell. The definition also includes, unless otherwise specifically indicated, chemically-modified amino acids, including amino acid analogues (such as penicillamine, 3-

mercapto-D-valine), naturally-occurring non-proteogenic amino acids (such as norleucine), beta-amino acids, azapeptides, N-methylated amino acids and chemically-synthesised compounds that have properties known in the art to be characteristic of an amino acid. The term "proteogenic" indicates that the amino acid can be incorporated into a protein in a cell through well-known metabolic pathways. The definition also includes amino acids in which the functional side group has been chemically derivatised. Such derivatised molecules include, for example, those molecules in which free amino groups have been derivatised to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatised to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatised to form O-acyl or O-alkyl derivatives. Also included as derivatives are those peptide portions that contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids.

Accordingly, it is appreciated that the peptide portions of the agent of the invention can be peptide "mimetics", i.e. peptidomimetics which mimic the structural features of peptides comprising or consisting of the amino acid sequence as described herein. Peptidomimetics can be even more advantageous in therapeutic use, in the resistance to degradation, in permeability or in possible oral administration.

A primary goal in the design of peptide mimetics has been to reduce the susceptibility of mimetics to cleavage and inactivation by peptidases. In one approach, such as disclosed by Sherman *et al* (1990), one or more amide bonds have been replaced in an essentially isosteric manner by a variety of chemical functional groups. This stepwise approach has met with some success in that active analogues have been obtained. In some instances, these analogues have been shown to possess longer biological half-lives than their naturally-occurring counterparts. In another approach, a variety of uncoded or modified amino acids such as D-amino acids and N-methyl amino acids have been used to modify mammalian peptides. Alternatively, a presumed bioactive conformation has been stabilised by a covalent modification, such as cyclization or by incorporation of γ-lactam or other types of bridges (Veber *et al*, 1978) and Thorsett *et al*, 1983). Another approach, disclosed by Rich (1986) has been to design peptide mimics through the application of the transition state analogue concept in enzyme inhibitor design. For example, it is known that the secondary alcohol of statine mimics the tetrahedral transition state of the sessile amide bond of the pepsin substrate. Other approaches include the use of azapeptides and beta-amino acids.

Also included in the definition of 'peptidomimetics', are retro-inverso peptides. By retro-inverso peptides (also known as all-D-retro or retro-enantio peptides) we include the meaning of a peptide in which all of the L-amino acids are replaced with D-amino acids and the peptide bonds are reversed. Thus, the peptides are composed of D-amino acids assembled in the reverse order from that of the parent L-sequence. Retro-inverso peptides can be synthesised by methods known in the art, for example such as those described in Meziere *et al* (1997) *J. Immunol.* **159** 3230-3237. This approach involves making pseudopeptides containing changes involving the backbone, and not the orientation of side chains which remain very similar to the parent peptide. Retro-inverse peptides are much more resistant to proteolysis.

Therefore, it will be appreciated that when any of the binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, T cell antigen, and spacer moieties as described herein are peptides or polypeptides, any one or more of those peptides or polypeptides may be substituted for a corresponding peptidomimetic that retains the respective activity of the parent peptide or polypeptide. This may help to confer protease resistance on the agent of the invention and thereby improve its stability. Thus, for example, when a T cell antigen is attached to a binding partner of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 via one or more peptide spacer moieties, it may be desirable for one or more of those spacer moieties to be peptidomimetics, e.g. wherein one or more of the naturally occurring amino acids of the spacer moieties are replaced or modified, for example, to improve stability.

Another approach to increase stability of peptide portions of the agent of the invention is to have stabilising groups at one or both termini. Typical stabilising groups include amido, acetyl, benzyl, phenyl, tosyl, alkoxycarbonyl, alkyl carbonyl, benzyloxycarbonyl and the like end group modifications. Additional modifications include using a "D" amino acid in place of a "L" amino acid at the termini, and amide rather than amino or carboxy termini or acetyl rather than amino termini, to inhibit exopeptidase activity. Thus, it is appreciated that whenever the agent of the invention has an exposed peptide terminus, that terminus may have a capping moiety, preferably a moiety that is less than 200 Da in molecular weight. Further capping moieties include a naftyl group or a polyethylene glycol group. It is appreciated that retro-inverso peptides are already relatively stable and so may not require additional capping moieties.

Preferably, the agent of the invention has a half-life in plasma of at least 24 hours at 37°C.

It may be desirable to modify the agent of the invention so that it can be more easily detected, for example by biotinylating it or by incorporating any detectable label known in the art such as radiolabels, fluorescent labels or enzymatic labels.

As described above, the inventors have shown that agents of the invention may be used to redirect existing immune responses to kill particular cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 in a specific manner. Since cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 are often cells which, at least in part, mediate the pathology of a biological or medical condition or disorder, the agents of the invention offer significant therapeutic potential.

Accordingly, a second aspect of the invention provides an agent according to the first aspect of the invention for use in medicine.

A third aspect of the invention also provides a pharmaceutical composition, comprising an agent according to the first aspect of the invention and a pharmaceutically acceptable carrier, diluent or excipient.

A fourth aspect of the invention provides a method of preventing or treating a condition characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, the method comprising administering an agent according to the first aspect of the invention. In this way, the agent of the invention will bind to the cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95, and, following internalisation, the T cell antigen will be presented on the surface of the cells making them a target for T cells. For the avoidance of doubt, an agent that comprises a binding partner for a particular target (eg CD70) will be used to prevent or treat a condition characterised by the presence of cells expressing that particular target (eg CD70).

Thus, the method may involve identifying a subject who has a condition or who is at risk of developing a condition characterised by any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 (eg cancer),

administering the agent according to the first aspect of the invention to the subject, and monitoring the levels of the cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 in the subject either by conducting tests to determine the number of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 or by monitoring the clinical symptoms of the subject. Depending on the results of the monitoring step, it may be necessary to administer more of the agent.

The invention includes an agent according to the first aspect of the invention for use in preventing or treating a condition characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95.

The invention includes the use of an agent according to the first aspect of the invention in the preparation of a medicament for preventing or treating a condition characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95.

By a 'condition characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95' we include any biological or medical condition or disorder in which at least part of the pathology is mediated by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95. The condition may be caused by the presence of the cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 or else the presence of the cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 may be an effect of the condition.

CD70 and CD74 are generally expressed by B cells, and so typically the condition is one where at least part of the pathology is mediated by B cells. For example, CD70 (CD27L) is a member of the tumour necrosis factor family aberrantly expressed on a number of hematologic malignancies and some carcinomas (eg renal cell carcinoma; Jilaveanu *et al*, *Human Palhol* **43(9)**: 1394). CD74 is expressed in parallel with MHC Class-II molecules and so is expressed on professional antigen presenting cells such as B-cells, monocytes, macrophages and dendritic cells. The condition may be one that affects one or more of these cells.

Cells expressing CD70 or CD74 are frequently implicated in lymphoma and several types of carcinoma. Thus, it is particularly preferred if the condition is a tumour (eg a malignant disease) and the cells expressing CD70 or CD74 are tumour cells or tumour associated tissue (eg tumour fibroblasts or tumour blood vessels). The condition may be any cancer such as breast cancer, ovarian cancer, endometrial cancer, cervical cancer, bladder cancer, renal cancer, melanoma, lung cancer, prostate cancer, testicular cancer, thyroid cancer, brain cancer, oesophageal cancer, gastric cancer, pancreatic cancer, colorectal cancer, liver cancer, leukaemia, myeloma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, acute myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphoblastic leukaemia, lymphoproliferative disorder, myelodysplastic disorder, myeloproliferative disease and premalignant disease.

Cells expressing CD74 have also been associated with allergic and autoimmune disease, and so in a further preferred embodiment when the binding partner is for CD74, the condition is allergic or autoimmune disease. Examples include rheumatoid arthritis, systemic lupus erythematosus and immune thrombocytopenia purpura.

Conditions characterised by the presence of cells expressing any of the other targets disclosed herein, include those mentioned in the above discussion of those targets.

The table below provides cellular expression data for each of the targets disclosed herein and conditions associated with each target. Thus, when the agent of the invention comprises a binding partner for a particular target, the agent may be used to prevent or treat one of the conditions associated with that target as set out in the table below.

| Target | Cellular Expression Data | Diseases associated with Target |
|---|---|---|
| HLA-DR | B-cells | B-cell Lymphomas (e.g. Hodgkin Lymphoma, Non-Hodgkin Lymphoma, B-cell chronic lymphocytic leukaemia) |
| | B-cells | Autoimmune diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus, autoimmune cytopenias) |
| | Myeloid Cells (Macrophages, Kupfer cells) | Myeloid Leukaemias |
| | Antigen Presenting cells (Dendritic Cells, Langerhans cells) | |

| | | |
|---|---|---|
| | Plasma Cells | Myeloma, Amyloid, Plasmacytomas |
| | | Autoimmune diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus, autoimmune cytopenias) |
| | | |
| CD74 | B-cells | B-cell Lymphomas (e.g. Hodgkin Lymphoma, Non-Hodgkin Lymphoma) |
| | B-cells | Autoimmune diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus, autoimmune cytopenias) |
| | Myeloid Cells (Macrophages, Kupfer cells) | Myeloid Leukaemias |
| | Antigen Presenting cells (Dendritic Cells, Langerhans cells) | |
| | Plasma Cells | Myeloma, Amyloid, Plasmacytomas |
| | | Autoimmune diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus, autoimmune cytopenias) |
| | | |
| CD22 | B-cells | B-cell Lymphomas (e.g. Hodgkin Lymphoma, Non-Hodgkin Lymphoma, B-cell chronic lymphocytic leukaemia) |
| | B-cells | Autoimmune diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus, autoimmune cytopenias) |
| | | |
| | | |
| CD23 | B-cells | B-cell Lymphomas (e.g. Hodgkin Lymphoma, Non-Hodgkin Lymphoma, B-cell chronic lymphocytic leukaemia) |
| | B-cells | Autoimmune diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus, autoimmune cytopenias) |
| CD43 | T-cells | Autoimmune diseases (e.g. diabetes melitus, anutoimmune hepatitis). |
| | Monocytes | |
| | B-cells | B-cell lymphoblastic lymphoma, Mucosa associated lymphoid tissue lymphoma |

| | | |
|---|---|---|
| | | |
| CD44 | Cancer Stem Cells | Many types of cancer including Breast, Colorectal, Ovarian, Head and Neck, leukaemias and gastrointestinal carcinomas. |
| | Squamous Cell Carcinoma | Head and Neck Cancers |
| | | |
| CD47 | Wide tissue expression | |
| | | |
| | | |
| CD54 | Vacular Endothelium | Vasculitis, Kaposi's sarcoma |
| | T-cells | Autoimmune diseases (e.g. diabetes melitus, anutoimmune hepatitis). |
| | B-cells | B-cell lymphoblastic lymphoma, Mucosa associated lymphoid tissue lymphoma |
| | | |
| CD58 | Vacular Endothelium | Vasculitis, Kaposi's sarcoma |
| | T-cells | Autoimmune diseases (e.g. diabetes melitus, anutoimmune hepatitis). |
| | B-cells | B-cell lymphoblastic lymphoma, Mucosa associated lymphoid tissue lymphoma |
| | | |
| CD55 | Widespread Tissue expression (Blood and Epithelia) | |
| | | |
| | | |
| CD59 | Widespread Tissue expression (Blood and Epithelia) | |
| | | |
| | | |
| CD62L | B-cells | Chronic lymphocytic leukaemia, |
| | T-cells | Adult T cell leukaemia |
| CD95 | Ubiquitous - can be upregulated on many cell types | Many including ovarian, liver and colorectal carcinoma, |
| | | |
| | | |
| CD30 | Activated T cells | Anaplastic large cell lymphoma & embryonal carcinoma |
| | | |
| | Activated B cells | Classical Hodgkin lymphoma |
| | | |

| | | |
|---|---|---|
| CD70 | B Cells | Hodgkin lymphoma, Non-Hodgkin lymphoma |
| | | |
| | Kidney | Renal cell carcinoma |
| | | Also pancreatic (25%), larynx/pharynx (22%), melanoma (16%), ovarian (15%), lung (10%), and colon (9%) |

Other conditions characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 can be readily determined by the skilled person. For example, the expression profile of any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 on cells may be carried out on a biopsy sample (eg from a cancer patient) using routine assays for measuring nucleic acid (e.g. DNA or RNA transcripts) or protein levels. Transcriptomic or proteomic techniques may be used. Also, immunohistochemistry and immunofluorescence may be used to quantitate antigen expression in tissues.

By preventing or treating a condition we include the meaning of reducing or alleviating symptoms in a patient (i.e. palliative use), preventing symptoms from worsening or progressing, treating the disorder (e.g. by inhibition or elimination of the causative agent), or prevention of the condition or disorder in a subject who is free therefrom.

It will be appreciated that the agents of the invention lend themselves to personalised medicine in the clinic whereby the most appropriate agent to be administered to the patient is determined, and either selected or prepared in the clinic. For example, before the step of administering the agent to the subject, any of the following may be determined: (i) the MHC alleles of the subject and/or (ii) the T cell response (eg cytotoxic T cells response) of the subject to a T cell antigen. The MHC alleles of a subject can be assessed by serological assays at the antigen level or by using DNA assays at the genetic level. Determining whether a given antigen stimulates a specific T cell response (eg cytotoxic T cell response) in a subject can be done by contacting isolated peripheral mononuclear blood cells from the subject with the antigen and using standard assays for cell proliferation.

Thus the method of the fourth aspect of the invention may include the steps of (i) identifying a subject who has a condition, or who is at risk of developing a condition

characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 (eg cancer), (ii) taking a sample from the subject, (iii) analysing the sample to identify the optimum T cell antigen preventing or treating the condition in that subject, (iii) preparing the agent of the invention, (iv) administering the agent to the subject, and (v) monitoring the levels of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 in the subject either by conducting tests to determine the number of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 or by monitoring the clinical symptoms of the subject.

It is appreciated that an apparatus may be used to select and optionally prepare the most appropriate agent to be used for a particular patient. For example, the apparatus may perform an automated analysis of one or more samples from the subject, and based on this analysis select and optionally prepare a tailor-made agent for that subject. Thus the apparatus may perform serological assays on the sample to determine a subject's MHC alleles and based on this test various peptides for their efficiency in eliciting a T cell response (eg cytotoxic T cell response), so as to identify the best T cell antigen for use in that patient. Similarly, the apparatus may carry out an expression profile of cells from the subject (eg from a biopsy sample) so as to determine a suitable binding partner for any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95.

By performing any one or more of these steps in the clinic an agent tailored for a particular subject can be prepared. For example, the agent can contain a T cell antigen that is known to bind to patient's MHC molecules and elicit a strong T cell response.

In one embodiment, the subject is administered a further therapeutic agent in addition to the agent according to the first aspect of the invention. For example, when administering the agent to prevent or treat a particular condition, a further therapeutic agent known to be useful for combating that condition may be administered. As an example, when the agent is for treating cancer, a further anti-cancer agent (eg anti-neoplastic chemotherapy) may be administered to the subject alongside the agent of the invention. Similarly, the further therapeutic agent may be one that is known to have therapeutic application in allergic disease, inflammatory disease, regenerative medicine and neuroregenerative disease.

It is appreciated that the further therapeutic agent may be administered at the same time as the agent of the invention (i.e. simultaneous administration optionally in a co-formulation) or at a different time to the agent of the invention (i.e. sequential administration).

The further therapeutic agent may be any one or more of a vaccine; an immuno stimulatory drug; an anti-cancer agent; an agent inhibiting an antibody response against the agent of the invention; and/or a protease inhibitor.

For example, in order to boost the effector immune response against the particular T cell antigen used, it may be desirable to vaccinate the subject with the T cell antigen; and/or administer immunostimulating agents such as IL-2, IL-7, IFNα, GM-CSF, metformin, lenalidomide; and/or administer anti-immunoregulatory agents such as Ipilimumab; all of which may be considered as further therapeutic agents. Similarly, the further therapeutic agent may be a live virus such as CMV that is used to stimulate an immune response against the T cell antigen. This may be performed by blood transfusion for example.

It is also appreciated that if the subject is one to whom is administered immunosuppressive agents, that these immunosuppressive agents are withdrawn from the subject (e.g. by suspending treatment) when or before being administered the agent of the invention.

Similarly, it may be desirable to employ methods aimed at circumventing any immunogenicity issues relating to the agent of the invention whereby an adverse antibody response is elicited *in vivo*. For example, the subject may also be administered one or more agents that are known to inhibit the activity of B cells, such as any of Rituximab, cyclophosphamide, Syk inhibitors, an anti-BAFF antibody (eg Belimumab), an anti-CD22 antibody, an anti-CD20 antibody and an anti-CD19 antibody, all of which may be considered as further therapeutic agents. In this case, it is particularly preferred if the inhibitor of B cells is administered to the subject prior to the agent of the invention, eg as a pre-treatment to ablate B cells.

The invention thus includes a composition comprising (i) an agent according to the first aspect of the invention and (ii) a further therapeutic agent, for use in preventing or treating a condition characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95. Given that the agent of the invention and the further therapeutic agent

may be administered simultaneously or sequentially, it will be appreciated that the invention includes an agent according to the first aspect of the invention for use in preventing or treating a condition characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 in a subject who is administered a further therapeutic agent. It also follows that the invention includes a therapeutic agent for use in preventing or treating a condition characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 in a subject who is administered an agent according to the first aspect of the invention.

Similarly, the invention includes a use of a composition comprising (i) an agent according to the first aspect of the invention and (ii) a further therapeutic agent, in the manufacture of a medicament for preventing or treating a condition characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95. Again, given that the agent of the invention and the further therapeutic agent may be administered simultaneously or sequentially, it will be appreciated that the invention includes the use of a composition comprising an agent according to the first aspect of the invention in the manufacture of a medicament for preventing or treating a condition characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 in a subject who is administered a further therapeutic agent. It also follows that the invention includes the use of a therapeutic agent in the manufacture of a medicament for preventing or treating a condition characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 in a subject who is administered an agent according to the first aspect of the invention.

The invention also provides a composition comprising (i) an agent according to the first aspect of the invention and (ii) a further therapeutic agent suitable for preventing or treating the same condition characterised by the presence of cells expressing any of CD70, CD74, CD22, HLA-DR, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95. It is appreciated that the therapeutic agent mentioned in the immediately preceding two paragraphs may be agents suitable for treating the same condition characterised by the presence of unwanted cells, as treatable by the agents of the invention.

Whilst it is possible for the agent of the invention to be administered alone, it is preferable to present it as a pharmaceutical formulation, together with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being compatible with the therapeutic agent and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free.

Where appropriate, the formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient (agent for treating or preventing a condition characterised by unwanted cells) with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide desired release profile.

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.

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. The formulations 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.

The agent 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, ointment or dusting powder. The agent may also be transdermally administered, for example, by the use of a skin patch.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

The amount of the agent which is administered to the individual is an amount effective to combat the particular individual's condition. The amount may be determined by the physician.

Preferably, in the context of any aspect of the invention described herein, the subject to be treated is a human. Alternatively, the subject may be an animal, for example a domesticated animal (for example a dog or cat), laboratory animal (for example laboratory rodent, for example mouse, rat or rabbit) or an animal important in agriculture (i.e. livestock), for example horses, cattle, sheep or goats.

In a preferred embodiment of the invention, the T cell antigen in the agent is a peptide, and the agent is used to prevent or treat cancer.

In an embodiment, the binding partner of CD70 is an anti-CD70 antibody such as BU69 antibody (anti-CD70, Birmingham University) (Leucocyte Typing V (1995): edited by SF Schlossman, OUP, Oxford) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD74 is an anti-CD74 antibody such as anti-CD74 (eBiosciences) anti-human CD74 purified, Clone: LN2) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD70 is an anti-CD70 antibody such as BU69 antibody (anti-CD70, Birmingham University) (Leucocyte Typing V (1995): edited by SF Schlossman, OUP, Oxford) and the T cell antigen comprises PRSPTVFYNIPPMPLPPSQL (SEQ ID No: 49).

In an embodiment, the binding partner of CD22 is an anti-CD22 antibody such as anti-CD22 (BD Bioscience) anti-human CD22 purified, Clone: HIB22) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD23 is an anti-CD23 antibody such as anti-CD23 (BD Bioscience) anti-human CD23 purified, Clone: M-L233) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD30 is an anti-CD30 antibody such as anti-CD30 (BD Bioscience) anti-human CD30 purified, Clone: BerH8) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD43 is an anti-CD43 antibody such as anti-CD43 (BD Bioscience) anti-human CD43 purified, Clone: 1G10) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD44 is an anti-CD44 antibody such as anti-CD44 (BD Bioscience) anti-human CD44 purified, Clone: 515) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD47 is an anti-CD47 antibody such as anti-CD47 (BD Bioscience) anti-human CD47 purified, Clone: B6H12) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD54 is an anti-CD54 antibody such as anti-CD54 (BD Bioscience) anti-human CD54 purified, Clone: 28/CD54) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD55 is an anti-CD55 antibody such as anti-CD55 (BD Bioscience) anti-human CD55 purified, Clone: IA10) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD58 is an anti-CD58 antibody such as anti-CD58 (BD Bioscience) anti-human CD58 purified, Clone: 1C3 (AICD58.6)) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD59 is an anti-CD59 antibody such as anti-CD59 (BD Bioscience) anti-human CD59 purified, Clone: p282 (H19)) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD62L is an anti-CD62L antibody such as anti-CD62L (BD Bioscience) anti-human CD62L purified, Clone: SK11) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of CD95 is an anti-CD95 antibody such as anti-CD95 (BD Bioscience) anti-human CD95 purified, Clone: EOS9.1) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

In an embodiment, the binding partner of HLA-DR is an anti-HLA-DR antibody such as anti-HLA-DR (BD Bioscience) anti-human HLA-DR purified, Clone: G46-6) and the T cell antigen comprises DYSNTHSTRYV (SEQ ID No: 9) or PDDYSNTHSTRYV (SEQ ID No: 108).

The invention will be described in further detail with the aid of the following Figures and Examples.

**FIGURE 1:** Amino acid sequences of human CD70, CD74, HLA-DR, CD22, CD23, CD30, CD43, CD44, CD47, CD54, CD55, CD58, CD59, CD62L or CD95 (SEQ ID Nos: 93-107).

**FIGURE 2:** Data demonstrating *in vitro* activity of redirected virus-specific T cells after internalisation of antibody peptide-epitope conjugate

(A) Recognition of a lymphoblastoid cell line by CD4+ cytomegalovirus-specific T cells through conjugation of the PDDYSNTHSTRYV (SEQ ID No: 108) peptide to BU69 antibody (anti-CD70, Birmingham University). Recognition of target cells is only present when the antibody is conjugated with the specific peptide. Controls demonstrate specificity of T cells for target cells only in the presence of exogenous peptide.

(B) Cytotoxicity of target cells labelled with BU69 conjugated with the viral peptide DYSNTHSTRYV at a ratio of 5 T cells to 1 target cell. The peptide-conjugated BU69 antibody mediates 40% cytotoxicity compared with 55% cytotoxicity of target cells exogenously pulsed with the viral peptide. There was very little toxicity seen when the BU69 antibody was conjugated with an irrelevant viral peptide.

(C) Recognition of a lymphoblastoid cell line by CD4+ cytomegalovirus-specific T cells through conjugation of the PDDYSNTHSTRYV peptide to anti-CD74 antibody (eBiosciences). Recognition of target cells is only present when the antibody is conjugated with the specific peptide. Controls demonstrate specificity of T cells for target cells only in the presence of exogenous peptide.

(D) Recognition of a lymphoblastoid cell line by CD4+ cytomegalovirus-specific T cells through conjugation of the PDDYSNTHSTRYV peptide to BU69 antibody in presence or absence of the heterobifunctional cross-linker sulfo-SMCC. Recognition of target cells is only present when the antibody is conjugated in the presence of the cross-linker compared with a lack of response when the cross-linker is not present. Controls demonstrate specificity of T cells for target cells only in the presence of exogenous peptide.

(E) Recognition of a lymphoblastoid cell line by CD4+ cytomegalovirus-specific T cells through conjugation of the PDDYSNTHSTRYV peptide to BU69 antibody in presence or absence of inhibitors of the MHC class I or class II antigen processing pathways. Recognition of target cells is reduced only when the cells are cultured in the presence of inhibitors of the MHC class II processing pathway (Chloroquine and Monensin) compared with the untreated control (PDDYSNTHSTRYV). There is no difference in the

T cell response towards cells cultured in the presence of inhibitors of the MHC class I processing pathway.

(F) Recognition of a lymphoblastoid cell line by CD4+ Epstein Barr virus-specific T cells through conjugation of the cognate antigen PRSPTVFYNIPPMPLPPSQL peptide to BU69 antibody (anti-CD70, Birmingham University). Recognition of target cells is only present when the antibody is conjugated with the specific peptide. Controls demonstrate specificity of T cells for target cells only in the presence of exogenous peptide.

(G-S) Recognition of a lymphoblastoid cell line by CD4+ cytomegalovirus-specific T cells through recognition of the peptide antigen PDDYSNTHSTRYV conjugated to a secondary antibody. Target cells were first labelled with a primary antibody that could bind to proteins expressed on the surface of the target cells (e.g. CD22, CD23, HLA-DR etc). A secondary antibody (anti-mouse IgG) conjugated with the peptide PDDYSNTHSTRYV is then used to label the antibody bound to the target cells. Target cells labelled with the APEC complex are recognised by peptide-specific T cells as determined by production of IFN-y. Controls demonstrate specificity of T cells for target cells only in the presence of exogenous peptide.

**Example 1: Stimulation of T cells by antibody peptide epitope conjugates (APECs)**

We have shown that by targeting T cell antigens to particular cell surface targets, the T cell antigen can be internalised and presented on the surface of the cell such that a T cell response is initiated.

Figure 2A demonstrates T cell recognition of target cells labelled with the agent. The anti-CD70 antibody is conjugated with a peptide (PDDYSNTHSTRYV) or without a peptide (DMSO) and used to label target cells. Cells labelled with the antibody without the peptide are not recognised by the T cells, demonstrated by the absence of IFN-y in the culture supernatant after overnight incubation of the target cells and the T cells. Cells labelled with the antibody conjugated with an immunogenic peptide are strongly recognised by the T cells due to the presence of IFN-y in the culture supernatant. These results suggest that the peptide has been released from the antibody and presented at the cell surface in complex with MHC class II molecules.

The control cells demonstrate that there is no IFN-y release by the target cells alone or in response to incubation with the immunogenic peptide. Also, there is no IFN-y release by T cells in the absence of the immunogenic peptide but once the target cells have been

labelled with exogenous immunogenic peptide, there is strong recognition of the T cells demonstrating that the T cells are peptide specific and do not recognise any other peptides naturally expressed by the target cell. Finally, there is no spontaneous release of IFN-y by the T cells throughout the time in culture.

In Figure 2B, target cells are labelled with an anti-CD70 APEC containing either a control peptide (Biotin-RPHERNFGTVL) or a test peptide (PDDYSNTHSTRYV). The labelled target cells are incubated with peptide-specific T cells for 6 hours and stained with an anti-CD20 antibody for flow cytometric analysis. Target cells labelled with the CD70-PDDYSNTHSTRYV APEC are recognised by the T cells and there is a reduction in the number of target cells left in the well after 6 hours compared with the target cells labelled with the Biotin-RPHERNFGTVL. The result here demonstrates an indirect method of T cell cytotoxicity directed against target cells labelled with APEC.

The control cells were either peptide pulsed or left untreated and cultured with T cells for 6 hours. Analysis on the flow cytometer demonstrated that target cells loaded with exogenous peptide were targeted by the T cells whereas target cells left untreated were ignored by the T cells.

In Figure 2C target cells are labelled with an anti-CD74 APEC containing either no peptide (DMSO), a control peptide (Biotin-RPHERNFGTVL) or the test peptide (PDDYSNTHSTRYV). After culturing with peptide-specific T cells, target cells labelled with the APEC containing the test peptide were recognised, as demonstrated by the release of IFN-y, whereas the target cells labelled with the APEC without a peptide or with a control peptide were not recognised.

In Figure 2D anti-CD70 APEC were generated, using DMSO as a no peptide control and the test peptide PDDYSNTHSTRYV, with or without the addition of sulfo-SMCC. This was to demonstrate that the conjugation of the peptide to the antibody is reliant on the hetero-bifunctional cross linker and not via any other chemical interaction. Target cells were labelled with the APEC and cultured with T cells for 16 hours. The supernatant of the cell culture was assayed for the presence of IFN-y. When the conjugation is done in the absence of sulfo-SMCC there is no recognition of the target cells labelled with the control or test APEC suggesting that there is no peptide presented at the surface of the cell. When the conjugation is done in the presence of sulfo-SMCC there is a T cell response to the target cells labelled with the test APEC (PDDYSNTHSTRYV) and no T cell response to the target cells labelled with the control APEC (DMSO). This result

demonstrates the requirement for SMCC during conjugation of the peptide to the antibody to generate the APEC.

In Figure 2E target cells were labelled with an anti-CD70- PDDYSNTHSTRYV APEC in the presence of inhibitors of the HLA class I and class II processing pathways. After addition of T cells to the labelled target cells and subsequent culture for 6 hours, the supernatant was assayed for the presence of IFN-y. Addition of lactacystin, pepstatin or 3-methyladenine (inhibitors of the class I processing pathway) demonstrate a similar level of T cell recognition compared to the cells cultured in the absence of inhibitors (PDDYSNTHSTRYV). Addition of monensin, chloroquine and leupeptin (inhibitors of various aspects of the class II processing pathway) demonstrate a decrease in the amount of IFN-y produced suggesting that the APEC is processed via the HLA class II processing pathway.

In Figure 2F anti-CD70 APEC were generated using the EBV-derived peptide PRSPTVFYNIPPMPLPPSQL and target cells labelled with the APEC. Peptide-specific T cells were added to the target cells and cultured together for 16 hours and the supernatant assayed for the presence of IFN-y. Target cells labelled with the test APEC (PRSPTVFYNIPPMPLPPSQL) were recognised by T cells whereas target cells labelled with control APEC (DMSO or Biotin-RPHERNFGTVL) were not recognised by the T cells.

Control cells pulsed with exogenous peptide were recognised strongly by the T cells where as untreated target cells were not recognised by the T cells. There was no spontaneous release of IFN-y by T cells when they were cultured alone.

In Figures 2G-S, anti-mouse IgG secondary antibody was conjugated with the CMV-derived peptide PDDYSNTHSTRYV. Target cells were first labelled with primary antibodies targeting various cell surface molecules and then labelled a second time using the peptide-conjugated secondary antibody. Peptide-specific T cells were added to the target cells and cultured together for 16 hours and the supernatant assayed for the presence of IFN-y. Target cells labelled with the test APEC after staining with the different primary antibodies were recognised by T cells.

Control cells pulsed with exogenous peptide were recognised strongly by the T cells where as untreated target cells were not recognised by the T cells. There was no spontaneous release of IFN-y by T cells when they were cultured alone.

**Example 2: Standard operating procedure for chemical conjugation of cysteinylated peptide to antibody**

1. Cysteinylated peptides dissolved in DMSO to final concentration of 10mg/ml.
2. Weigh 1mg Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (Sulfo-SMCC) and dissolve in 200µl phosphate buffered saline (PBS).
   a. Other heterobifunctional cross-linkers could be used in place of Sulfo-SMCC e.g. Sulfosuccinimidyl 6-(3'-[2-pyridyldithio]-propionamido) hexanoate (Sulfo-LC-SPDP) and N-[ß-Maleimidopropionic acid] hydrazide, trifluoroacetic acid salt (BMPH) amongst others.
3. Add 50µl antibody (10mg/ml, 500µg antibody) to dissolved Sulfo-SMCC and incubate at room temperature for 30 minutes.
4. Wash a ZebaSpin Desalting column (7kDa molecular weight) (Thermo Fisher) by firstly spinning the column at 1,500g for 1 minute to remove the ethanol (storage buffer).
5. Add 300µl PBS and spin at 1,500g for 1 minute. Remove eluate and repeat a further two times.
6. Add 125ul antibody-SMCC to column, mix well and incubate for 2 minutes.
7. To elute the bound antibody, centrifuge at 1,500g for 2 minute and collect eluate.
8. Add 5µl peptide, previously dissolved in DMSO, to the SMCC-activated antibody and incubate at room temperature for 30 minutes.
9. Wash a Protein G column (GE Healthcare) by firstly spinning the column at 13,000rpm for 30 seconds to remove the ethanol (storage buffer).
10. Add 500µl PBS and mix protein G beads well before spinning at 13,000rpm for 30 seconds. Remove eluate and repeat wash a further two times.
11. Add antibody-SMCC to protein G column, mix well and incubate for 5 minutes. Centrifuge at 13,000rpm for 30 seconds and remove eluate.
12. Wash antibody by adding 500µl PBS and mixing the beads well before spinning at 13,000rpm for 30 seconds and removing eluate. Repeat this step a further two times.
13. To elute the bound antibody, add 125µl 0.1M citric acid to the beads and incubate for 2 minutes at room temperature. Place column in a 1.5ml eppendorf and spin at 13,000rpm for 30 seconds and collect eluate.
14. Repeat elution a second time for total elution volume of 250ul.
15. Add 750µl 0.2M Na2HCO3 to increase the pH to ~7. Leave at room temperature for 10 minutes. Antibody-peptide conjugate can now be used to stain cells.
16. Store antibody at 4°C.

**CLAIMS**

1. An agent comprising:

(i) a T cell antigen, and

(ii) a binding partner for CD30,

wherein, following binding of the agent to a cell that expresses CD30, the agent is internalised and the T cell antigen is presented on the surface of the cell in a form that can be recognised by a T cell, and further wherein the agent does not comprise a cleavage site cleavable by an extracellular molecule.

2. An agent according to Claim 1, wherein the T cell antigen is attached to the binding partner for CD30, such that the T cell antigen can be released from the binding partner within the cell that expresses CD30.

3. An agent according to Claim 2, wherein the T cell antigen can be released from the binding partner by an intracellular protease.

4. An agent according Claim 2, wherein, following binding of the agent to a cell that expresses CD30, the agent is internalised and the T cell antigen is presented on the surface of the cell by binding to a MHC molecule or Group I CD1 molecule.

5. An agent according to any of Claims 1-4 wherein the binding partner for CD30, is any of an antibody, a hormone, a growth factor, a cytokine, or a receptor ligand.

6. An agent according to Claim 5, wherein the binding partner is an antibody.

7. An agent according to any of Claims 1-6, wherein the T cell antigen is one that is capable of eliciting an existing T cell response in a subject.

8. An agent according to any of Claims 1-7, wherein the T cell antigen is any of a peptide, a polypeptide, a phosphopeptide or a lipid such as a phospholipid or sphingolipid.

9. An agent according to Claim 7 or 8, wherein the antigen is a viral-derived antigen.

10. An agent according to any of Claims 1-9, wherein the antigen is derived from any of Epstein Barr virus (eg HHV4), cytomegalovirus (eg human cytomegalovirus), Varicella Zoster virus, Herpes simplex virus, adenovirus, rhinovirus, influenza virus, or derived from a vaccine such as tetanus toxoid.

11. An agent according to any of Claims 1-10, wherein the T cell antigen is an MHC Class II restricted antigen, or an antigen that is capable of binding to a group I CD1 molecule.

12. An agent according to any of Claims 1-11, for use in medicine.

13. A pharmaceutical composition, comprising an agent according to any of Claims 1-11, and a pharmaceutically acceptable carrier, diluent or excipient.

14. A method of preventing or treating a condition characterised by the presence of cells expressing CD30, the method comprising administering an agent according to any of Claims 1-11.

15. An agent according to any of Claims 1-11 for use in preventing or treating a condition characterised by the presence of cells expressing CD30,.

16. Use of an agent according to any of Claims 1-11 in the preparation of a medicament for preventing or treating a condition characterised by the presence of cells expressing CD30.

17. A method according to Claim 14, wherein before the step of administering the agent to the subject, one or both of (i) the MHC alleles of the subject, and (ii) the cytotoxic T cell response of the subject to a T cell antigen, is determined.

18. A method according to Claim 14 or 17, further comprising administering a further therapeutic agent to the subject.

19. A composition or kit of parts comprising (i) an agent according to any of Claims 1-11 and (ii) a further therapeutic agent.

20. An agent according to any of Claims 1-11 and a further therapeutic agent for use in preventing or treating a condition characterised by the presence of cells expressing CD30.

21. An agent according to any of Claims 1-11 for use in preventing or treating a condition characterised by the presence of cells expressing CD30, wherein the subject is also administered a further therapeutic agent.

22. A therapeutic agent for use in preventing or treating a condition characterised by the presence of cells expressing CD30 , wherein the subject is also administered an agent according to any of Claims 1-11.

23. Use of an agent according to any of Claims 1-11 and a further therapeutic agent in the preparation of a medicament for preventing or treating a condition characterised by the presence of cells expressing CD30.

24. Use of an agent according to any of Claims 1-11 in the preparation of a medicament for preventing or treating a condition characterised by the presence of cells expressing CD30, wherein the subject is also administered a further therapeutic agent.

25. Use of a therapeutic agent in the preparation of a medicament for preventing or treating a condition characterised by the presence of cells expressing CD30, wherein the subject is also administered an agent according to any of Claims 1-11.

26. An agent according to any of Claims 1-12, 15 and 20-22, a method according to any of Claims 14, 17 and 18, a composition or kit of parts according to Claim 13 or 19, or a use according to any of Claims 16 and 23-25, wherein the condition characterised by the presence of cells expressing CD30, is any of a tumour (benign or malignant) or an autoimmune condition.

27. An agent according to any of Claims 1-12, 15, 20-22 and 26, a method according to any of Claims 14, 17, 18 and 26, a composition or kit of parts according to any of Claims 13, 19 and 26, or a use according to any of Claims 16 and 23-26, wherein the condition characterised by the presence of cells expressing CD30, is a tumour, and the T cell antigen in the agent is a peptide.

28. A method according to any of Claims 18, 26 and 27, a composition or kit of parts according to any of Claims 19, 26 and 27, an agent according to any of Claims 20-22, 26 and 27, or a use according to any of Claims 23-27, wherein the therapeutic agent is one that is suitable for preventing or treating the condition characterised by the presence of cells expressing CD30.

29. A method according to any of Claims 18 and 26-28, a composition or kit of parts according to any of Claims 19 and 26-28, an agent according to any of Claims 20-22 and 26-28, or a use according to any of Claims 23-28, wherein the further therapeutic agent is any one

or more of a vaccine, an immunostimulatory drug, a live virus, an anti-cancer agent, an inhibitor of an antibody response against the agent of the invention, and a protease inhibitor.

# Figure 1 (Page 1 of 6)

## (A) Amino acid sequence of human CD70

MPEEGSGCSVRRRPYGCVLRAALVPLVAGLVICLVVCIQRFAQAQQQLPLESLGWDVAELQ
LNHTGPQQDPRLYWQGGPALGRSFLHGPELDKGQLRIHRDGIYMVHIQVTLAICSSTTASR
HHPTTLAVGICSPASRSISLLRLSFHQGCTIASQRLTPLARGDTLCTNLTGTLLPSRNTDETFF
GVQWVRP

## (B) Amino acid sequence of human CD74

MHRRRSRSCREDQKPVMDDQRDLISNNEQLPMLGRRPGAPESKCSRGALYTGFSILVTLL
LAGQATTAYFLYQQQGRLDKLTVTSQNLQLENLRMKLPKPPKPVSKMRMATPLLMQALPM
GALPQGPMQNATKYGNMTEDHVMHLLQNADPLKVYPPLKGSFPENLRHLKNTMETIDWKV
FESWMHHWLLFEMSRHSLEQKPTDAPPKVLTKCQEEVSHIPAVHPGSFRPKCDENGNYLP
LQCYGSIGYCWCVFPNGTEVPNTRSRGHHNCSESLELEDPSSGLGVTKQDLGPVPM

## (C) Amino acid sequence of human HLA-DR alpha

MAISGVPVLGFFIIAVLMSAQESWAIKEEHVIIQAEFYLNPDQSGEFMFDFDGDEIFHVD
MAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNYTPITNVPPEVTVLTNS
PVELREPNVLICFIDKFTPPVVNVTWLRNGKPVTTGVSETVFLPREDHLFRKFHYLPFLP
STEDVYDCRVEHWGLDEPLLKHWEFDAPSPLPETTENVVCALGLTVGLVGIIIGTIFIIK
GVRKSNAAERRGPL

## (D) Amino acid sequence of human CD22

Alpha Form

MHLLGPWLLLLVLEYLAFSDSSKWVFEHPETLYAWEGACVWIPCTYRALDGDLESFILFH
NPEYNKNTSKFDGTRLYESTKDGKVPSEQKRVQFLGDKNKNCTLSIHPVHLNDSGQLGLR
MESKTEKWMERIHLNVSERPFPPHIQLPPEIQESQEVTLTCLLNFSCYGYPIQLQWLLEG
VPMRQAAVTSTSLTIKSVFTRSELKFSPQWSHHGKIVTCQLQDADGKFLSNDTVQLNVKH
PPKKVTTVIQNPMPIREGDTVTLSCNYNSSNPSVTRYEWKPHGAWEEPSLGVLKIQNVGW
DNTTIACAACNSWCSWASPVALNVQYAPRDVRVRKIKPLSEIHSGNSVSLQCDFSSSHPK
EVQFFWEKNGRLLGKESQLNFDSISPEDAGSYSCWVNNSIGQTASKAWTLEVLYAPRRLR
VSMSPGDQVMEGKSATLTCESDANPPVSHYTWFDWNNQSLPYHSQKLRLEPVKVQHSGA
Y

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WCQGTNSVGKGRSPLSTLTVYYSPETIGRRVAVGLGSCLAILILAICGLKLQRRWKRTQS
QQGLQENSSGQSFFVRNKKVRRAPLSEGPHSLGCYNPMMEDGISYTTLRFPEMNIPRTGD
AESSEMQRPPPDCDDTVTYSALHKRQVGDYENVIPDFPEDEGIHYSELIQFGVGERPQAQ
ENVDYVILKH

Beta Form

MHLLGPWLLLLVLEYLAFSDSSKWVFEHPETLYAWEGACVWIPCTYRALDGDLESFILFH
NPEYNKNTSKFDGTRLYESTKDGKVPSEQKRVQFLGDKNKNCTLSIHPVHLNDSGQLGLR
MESKTEKWMERIHLNVSERPFPPHIQLPPEIQESQEVTLTCLLNFSCYGYPIQLQWLLEG
VPMRQAAVTSTSLTIKSVFTRSELKFSPQWSHHGKIVTCQLQDADGKFLSNDTVQLNVKH
TPKLEIKVTPSDAIVREGDSVTMTCEVSSSNPEYTTVSWLKDGTSLKKQNTFTLNLREVT
KDQSGKYCCQVSNDVGPGRSEEVFLQVQYAPEPSTVQILHSPAVEGSQVEFLCMSLANPL
PTNYTWYHNGKEMQGRTEEKVHIPKILPWHAGTYSCVAENILGTGQRGPGAELDVQYPPK
KVTTVIQNPMPIREGDTVTLSCNYNSSNPSVTRYEWKPHGAWEEPSLGVLKIQNVGWDNT
TIACAACNSWCSWASPVALNVQYAPRDVRVRKIKPLSEIHSGNSVSLQCDFSSSHPKEVQ
FFWEKNGRLLGKESQLNFDSISPEDAGSYSCWVNNSIGQTASKAWTLEVLYAPRRLRVSM
SPGDQVMEGKSATLTCESDANPPVSHYTWFDWNNQSLPYHSQKLRLEPVKVQHSGAYWC
Q

GTNSVGKGRSPLSTLTVYYSPETIGRRVAVGLGSCLAILILAICGLKLQRRWKRTQSQQG
LQENSSGQSFFVRNKKVRRAPLSEGPHSLGCYNPMMEDGISYTTLRFPEMNIPRTGDAES
SEMQRPPPDCDDTVTYSALHKRQVGDYENVIPDFPEDEGIHYSELIQFGVGERPQAQENV
DYVILKH

## (E) Amino acid sequence of human CD23

MEEGQYSEIEELPRRRCCRRGTQIVLLGLVTAALWAGLLTLLLLWHWDTTQSLKQLEERA
ARNVSQVSKNLESHHGDQMAQKSQSTQISQELEELRAEQQRLKSQDLELSWNLNGLQADL
SSFKSQELNERNEASDLLERLREEVTKLRMELQVSSGFVCNTCPEKWINFQRKCYYFGKG
TKQWVHARYACDDMEGQLVSIHSPEEQDFLTKHASHTGSWIGLRNLDLKGEFIWVDGSHV
DYSNWAPGEPTSRSQGEDCVMMRGSGRWNDAFCDRKLGAWVCDRLATCTPPASEGSAE
SMGPDSRPDPDGRLPTPSAPLHS

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## (F) Amino acid sequence of human CD30

MRVLLAALGLLFLGALRAFPQDRPFEDTCHGNPSHYYDKAVRRCCYRCPMGLFPTQQCPQ
RPTDCRKQCEPDYYLDEADRCTACVTCSRDDLVEKTPCAWNSSRVCECRPGMFCSTSAV
N
SCARCFFHSVCPAGMIVKFPGTAQKNTVCEPASPGVSPACASPENCKEPSSGTIPQAKPT
PVSPATSSASTMPVRGGTRLAQEAASKLTRAPDSPSSVGRPSSDPGLSPTQPCPEGSGDC
RKQCEPDYYLDEAGRCTACVSCSRDDLVEKTPCAWNSSRTCECRPGMICATSATNSCARC
VPYPICAAETVTKPQDMAEKDTTFEAPPLGTQPDCNPTPENGEAPASTSPTQSLLVDSQA
SKTLPIPTSAPVALSSTGKPVLDAGPVLFWVILVLVVVVGSSAFLLCHRRACRKRIRQKL
HLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGLMSQPLMETCHSVGAAYL
ESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVGTVKAELPEGRGL
AGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPTAASGK

## (G) Amino acid sequence of human CD43

MATLLLLLGVLVVSPDALGSTTAVQTPTSGEPLVSTSEPLSSKMYTTSITSDPKADSTGD
QTSALPPSTSINEGSPLWTSIGASTGSPLPEPTTYQEVSIKMSSVPQETPHATSHPAVPI
TANSLGSHTVTGGTITTNSPETSSRTSGAPVTTAASSLETSRGTSGPPLTMATVSLETSK
GTSGPPVTMATDSLETSTGTTGPPVTMTTGSLEPSSGASGPQVSSVKLSTMMSPTTSTNA
STVPFRNPDENSRGMLPVAVLVALLAVIVLVALLLLWRRRQKRRTGALVLSRGGKRNGVV
DAWAGPAQVPEEGAVTVTVGGSGGDKGSGFPDGEGSSRRPTLTTFFGRRKSRQGSLAME
E
LKSGSGPSLKGEEEPLVASEDGAVDAPAPDEPEGGDGAAP

## (H) Amino acid sequence of human CD44

MDKFWWHAAWGLCLVPLSLAQIDLNITCRFAGVFHVEKNGRYSISRTEAADLCKAFNSTL
PTMAQMEKALSIGFETCRYGFIEGHVVIPRIHPNSICAANNTGVYILTSNTSQYDTYCFN
ASAPPEEDCTSVTDLPNAFDGPITITIVNRDGTRYVQKGEYRTNPEDIYPSNPTDDDVSS
GSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTLMSTSATATETATKRQE
TWDWFSWLFLPSESKNHLHTTTQMAGTSSNTISAGWEPNEENEDERDRHLSFSGSGIDDD
EDFISSTISTTPRAFDHTKQNQDWTQWNPSHSNPEVLLQTTTRMTDVDRNGTTAYEGNWN
PEAHPPLIHHEHHEEEETPHSTSTIQATPSSTTEETATQKEQWFGNRWHEGYRQTPKEDS
HSTTGTAAASAHTSHPMQGRTTPSPEDSSWTDFFNPISHPMGRGHQAGRRMDMDSSHSIT

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LQPTANPNTGLVEDLDRTGPLSMTTQQSNSQSFSTSHEGLEEDKDHPTTSTLTSSNRNDV
TGGRRDPNHSEGSTTLLEGYTSHYPHTKESRTFIPVTSAKTGSFGVTAVTVGDSNSNVNR
SLSGDQDTFHPSGGSHTTHGSESDGHSHGSQEGGANTTSGPIRTPQIPEWLIILASLLAL
ALILAVCIAVNSRRRCGQKKKLVINSGNGAVEDRKPSGLNGEASKSQEMVHLVNKESSET
PDQFMTADETRNLQNVDMKIGV

## (I) Amino acid sequence of human CD47

MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKF
KGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELT
REGETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALL
VAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIA
ILVIQVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQ
PPRKAVEEPLNAFKESKGMMNDE

## (J) Amino acid sequence of human CD54

MAPSSPRPALPALLVLLGALFPGPGNAQTSVSPSKVILPRGGSVLVTCSTSCDQPKLLGI
ETPLPKKELLLPGNNRKVYELSNVQEDSQPMCYSNCPDGQSTAKTFLTVYWTPERVELAP
LPSWQPVGKNLTLRCQVEGGAPRANLTVVLLRGEKELKREPAVGEPAEVTTTVLVRRDHH
GANFSCRTELDLRPQGLELFENTSAPYQLQTFVLPATPPQLVSPRVLEVDTQGTVVCSLD
GLFPVSEAQVHLALGDQRLNPTVTYGNDSFSAKASVSVTAEDEGTQRLTCAVILGNQSQE
TLQTVTIYSFPAPNVILTKPEVSEGTEVTVKCEAHPRAKVTLNGVPAQPLGPRAQLLLKA
TPEDNGRSFSCSATLEVAGQLIHKNQTRELRVLYGPRLDERDCPGNWTWPENSQQTPMC
Q
AWGNPLPELKCLKDGTFPLPIGESVTVTRDLEGTYLCRARSTQGEVTRKVTVNVLSPRYE
IVIITVVAAAVIMGTAGLSTYLYNRQRKIKKYRLQQAQKGTPMKPNTQATPP

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## (K) Amino acid sequence of human CD55

MTVARPSVPAALPLLGELPRLLLLVLLCLPAVWGDCGLPPDVPNAQPALEGRTSFPEDTV
ITYKCEESFVKIPGEKDSVICLKGSQWSDIEEFCNRSCEVPTRLNSASLKQPYITQNYFP
VGTVVEYECRPGYRREPSLSPKLTCLQNLKWSTAVEFCKKKSCPNPGEIRNGQIDVPGGI
LFGATISFSCNTGYKLFGSTSSFCLISGSSVQWSDPLPECREIYCPAPPQIDNGIIQGER
DHYGYRQSVTYACNKGFTMIGEHSIYCTVNNDEGEWSGPPPECRGKSLTSKVPPTVQKPT
TVNVPTTEVSPTSQKTTTKTTTPNAQATRSTPVSRTTKHFHETTPNKGSGTTSGTTRLLS
GHTCFTLTGLLGTLVTMGLLT

## (L) Amino acid sequence of human CD58

MVAGSDAGRALGVLSVVCLLHCFGFISCFSQQIYGVVYGNVTFHVPSNVPLKEVLWKKQK
DKVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTMKFFLYV
LESLPSPTLTCALTNGSIEVQCMIPEHYNSHRGLIMYSWDCPMEQCKRNSTSIYFKMEND
LPQKIQCTLSNPLFNTTSSIILTTCIPSSGHSRHRYALIPIPLAVITTCIVLYMNGILKC
DRKPDRTNSN

(Isoform2)
MVAGSDAGRALGVLSVVCLLHCFGFISCFSQQIYGVVYGNVTFHVPSNVPLKEVLWKKQK
DKVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTMKFFLYV
LESLPSPTLTCALTNGSIEVQCMIPEHYNSHRGLIMYSWDCPMEQCKRNSTSIYFKMEND
LPQKIQCTLSNPLFNTTSSIILTTCIPSSGHSRHRYALIPIPLAVITTCIVLYMNVL

## (M) Amino acid sequence of human CD59

MGIQGGSVLFGLLLVLAVFCHSGHSLQCYNCPNPTADCKTAVNCSSDFDACLITKAGLQV
YNKCWKFEHCNFNDVTTRLRENELTYYCCKKDLCNFNEQLENGGTSLSEKTVLLLVTPFL
AAAWSLHP

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## **(N)** Amino acid sequence of human CD62L

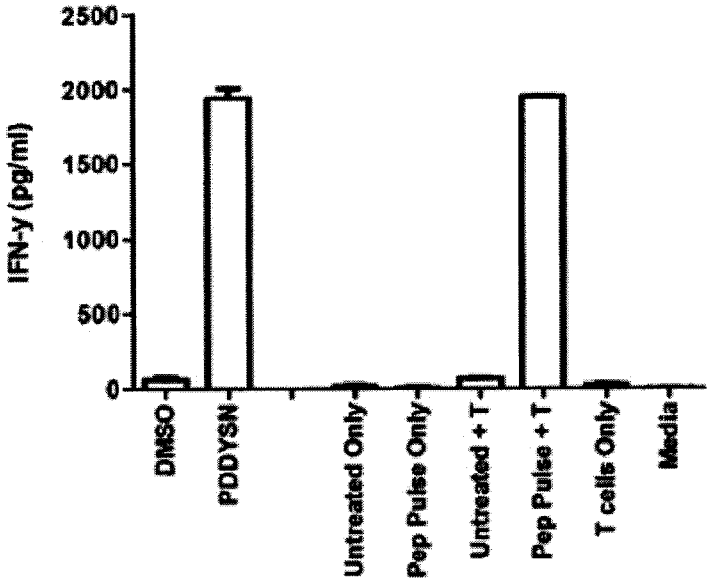
MIFPWKCQSTQRDLWNIFKLWGWTMLCCDFLAHHGTDCWTYHYSEKPMNWQRARRFCR
DN
YTDLVAIQNKAEIEYLEKTLPFSRSYYWIGIRKIGGIWTWVGTNKSLTEEAENWGDGEPN
NKKNKEDCVEIYIKRNKDAGKWNDDACHKLKAALCYTASCQPWSCSGHGECVEIINNYTC
NCDVGYYGPQCQFVIQCEPLEAPELGTMDCTHPLGNFSFSSQCAFSCSEGTNLTGIEETT
CGPFGNWSSPEPTCQVIQCEPLSAPDLGIMNCSHPLASFSFTSACTFICSEGTELIGKKK
TICESSGIWSNPSPICQKLDKSFSMIKEGDYNPLFIPVAVMVTAFSGLAFIIWLARRLKK
GKKSKRSMNDPY

## **(O)** Amino acid sequence of human CD95

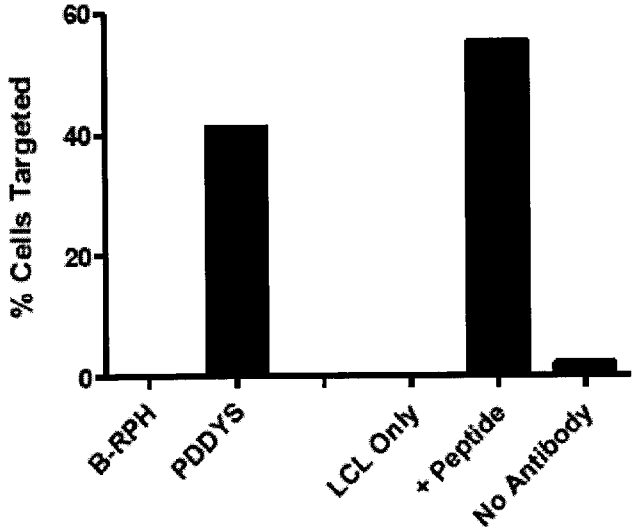
MLGIWTLLPLVLTSVARLSSKSVNAQVTDINSKGLELRKTVTTVETQNLEGLHHDGQFCH
KPCPPGERKARDCTVNGDEPDCVPCQEGKEYTDKAHFSSKCRRCRLCDEGHGLEVEINCT
RTQNTKCRCKPNFFCNSTVCEHCDPCTKCEHGIIKECTLTSNTKCKEEGSRSNLGWLCLL
LLPIPLIVWVKRKEVQKTCRKHRKENQGSHESPTLNPETVAINLSDVDLSKYITTIAGVM
TLSQVKGFVRKNGVNEAKIDEIKNDNVQDTAEQKVQLLRNWHQLHGKKEAYDTLIKDLKK
ANLCTLAEKIQTIILKDITSDSENSNFRNEIQSLV

# Figure 2 (Page 1 of 8)

A




B



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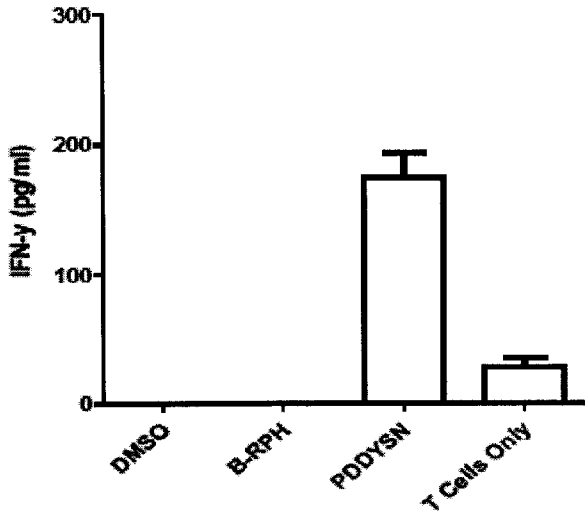
C

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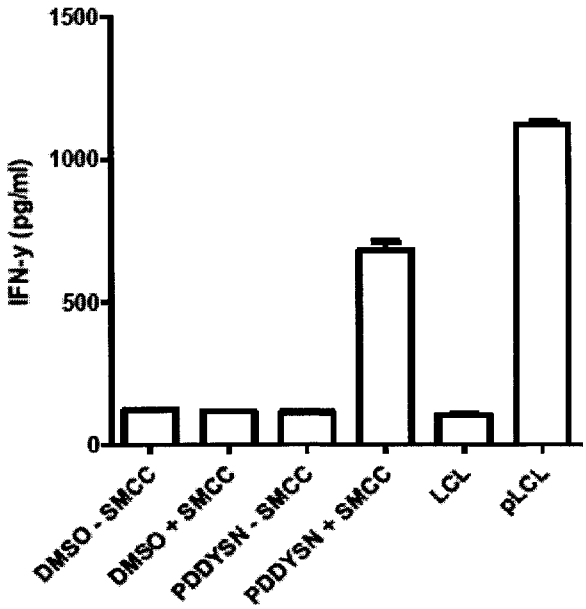

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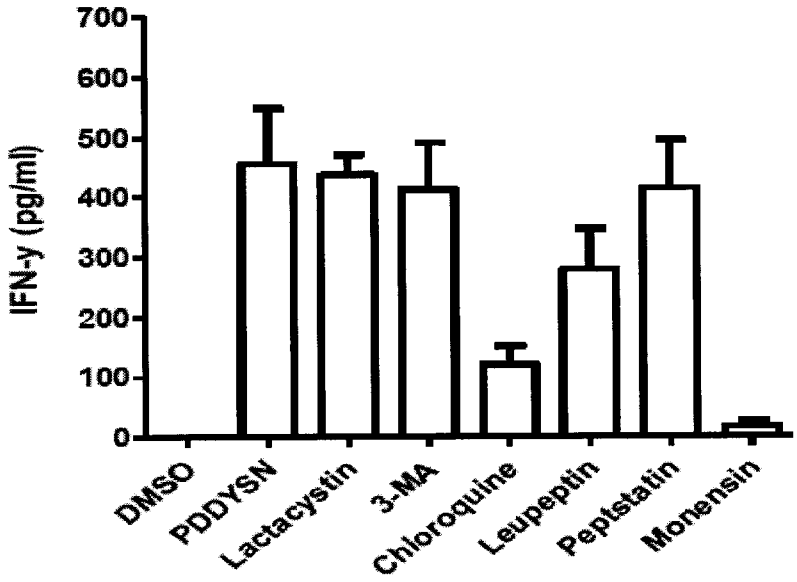
E

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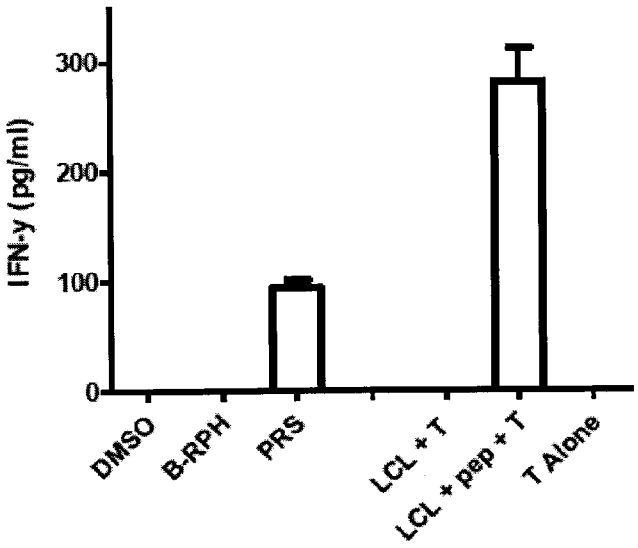

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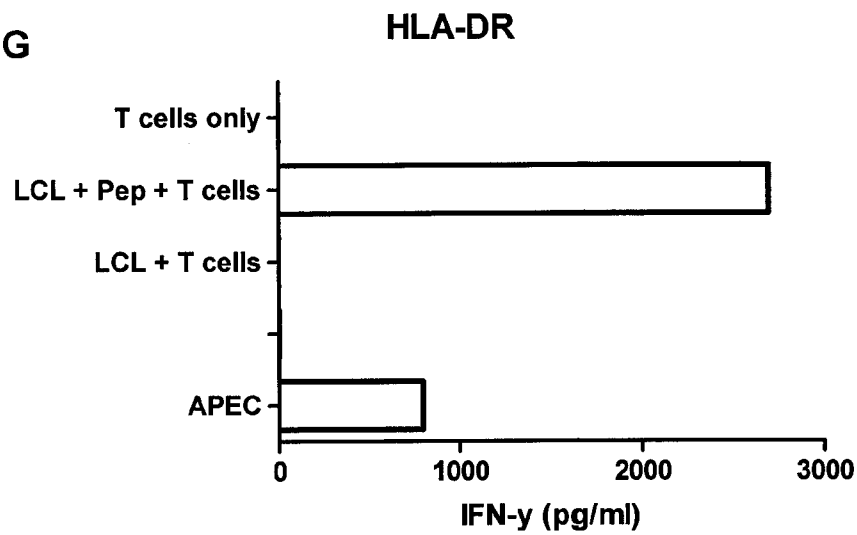
G

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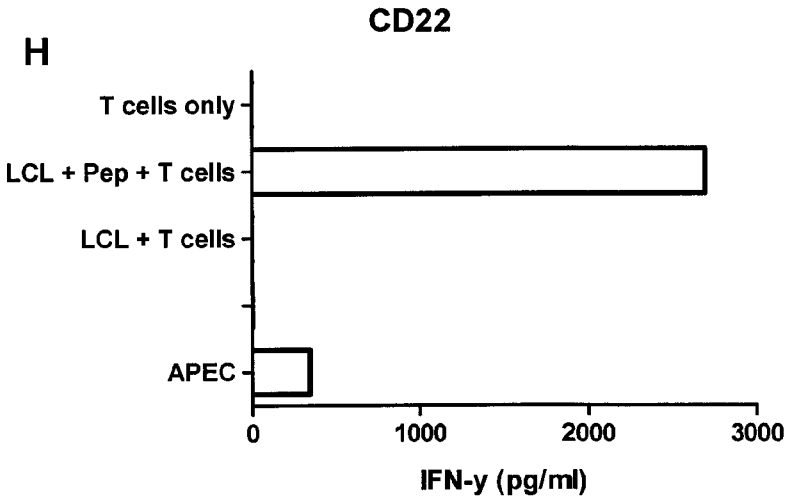


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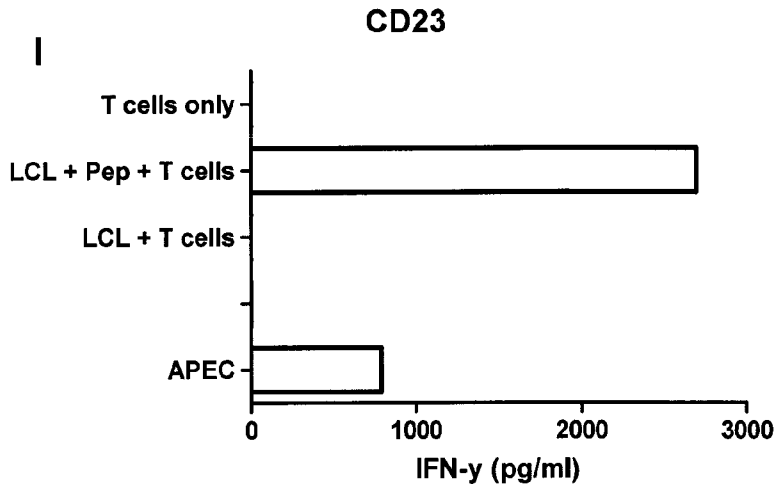

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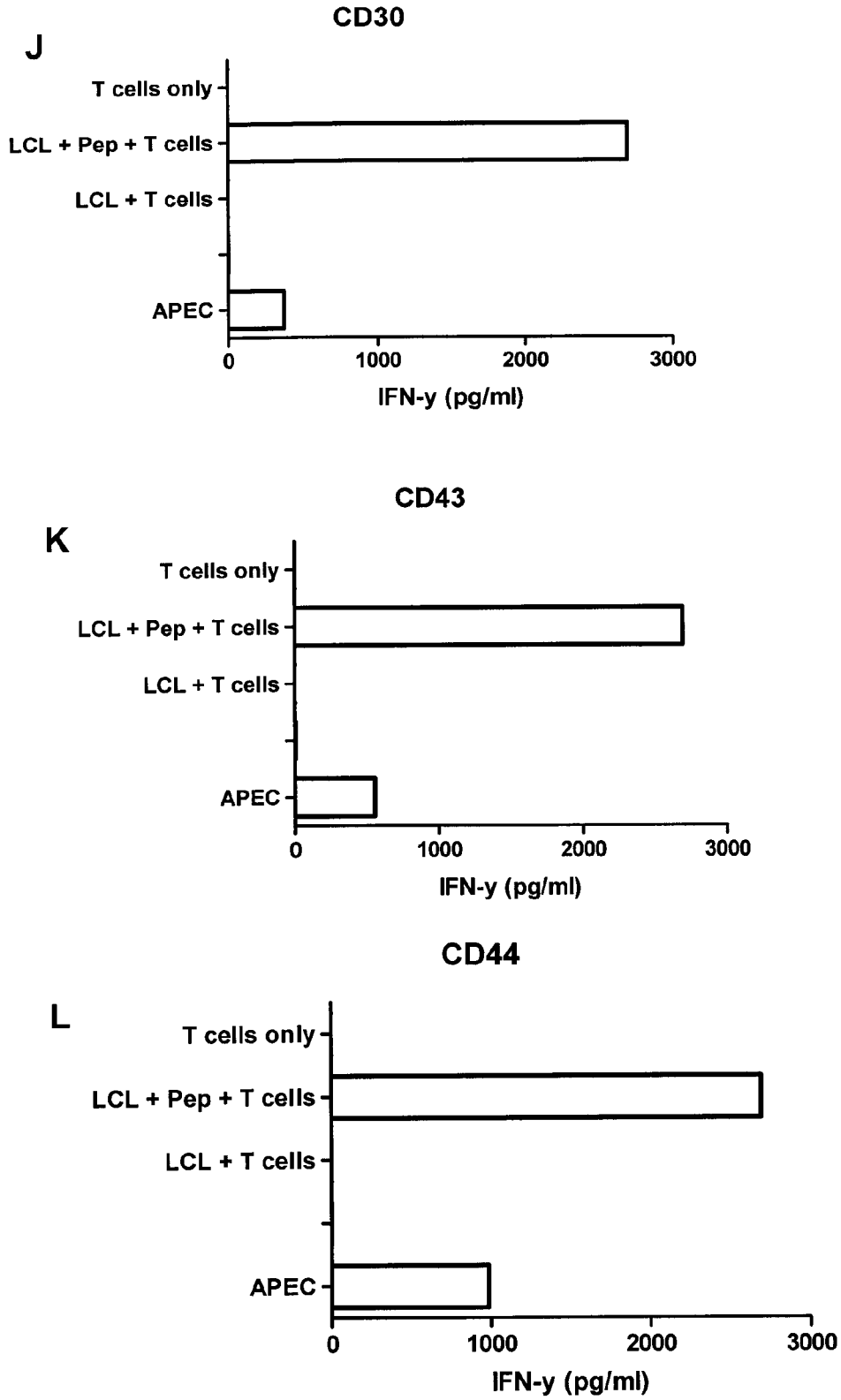

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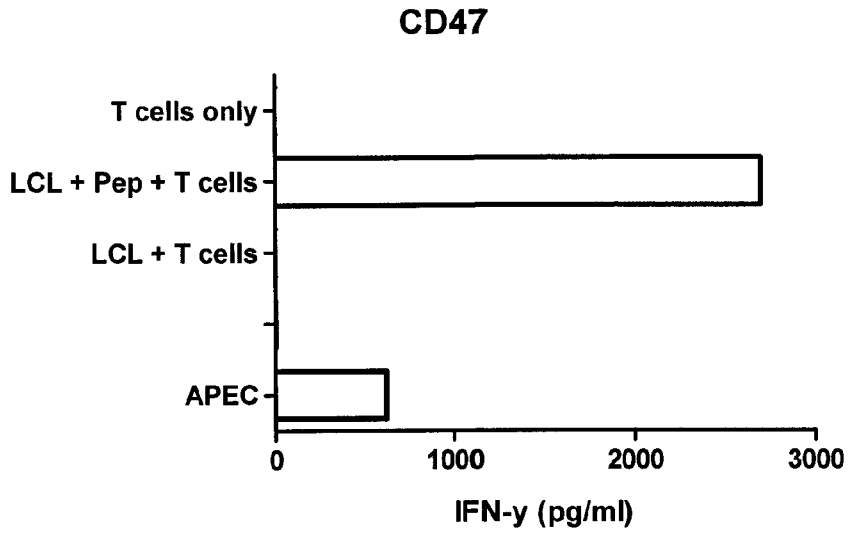
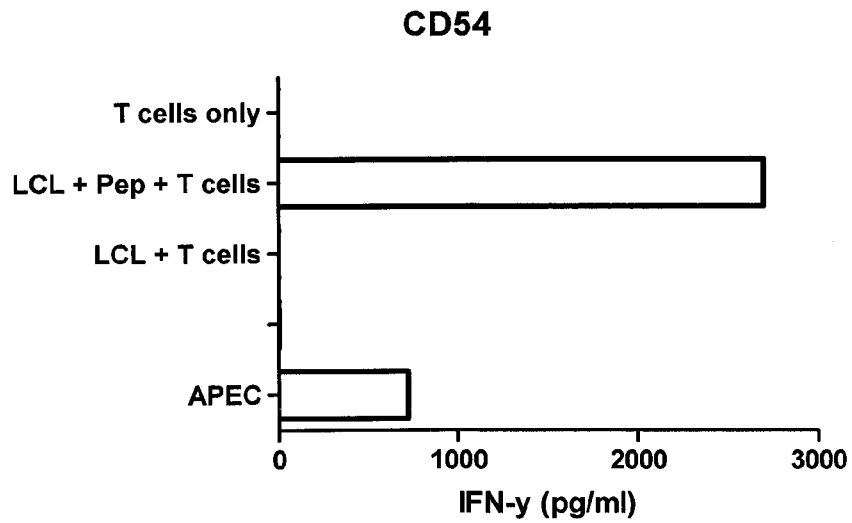
M




N



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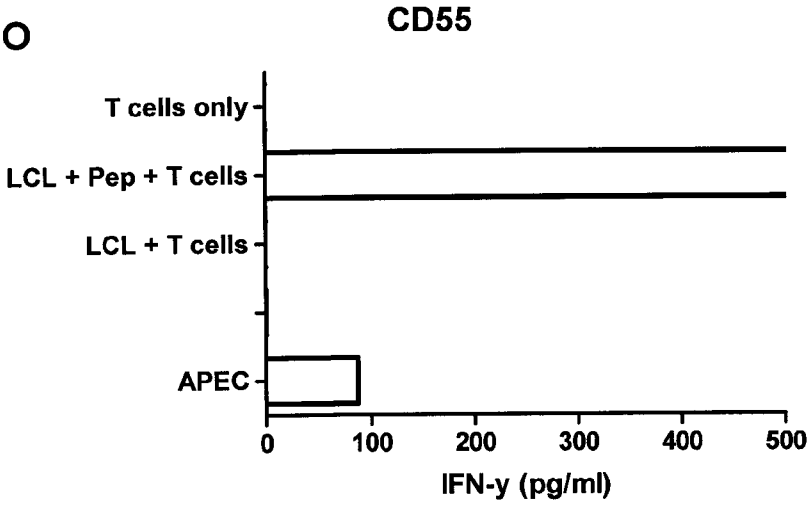
O

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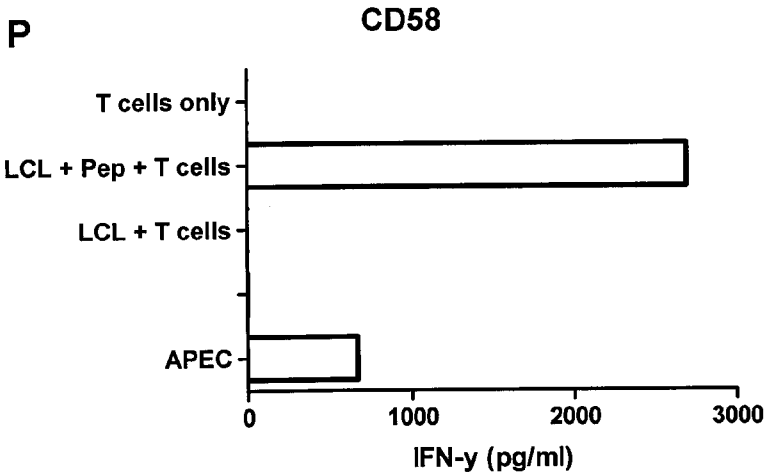


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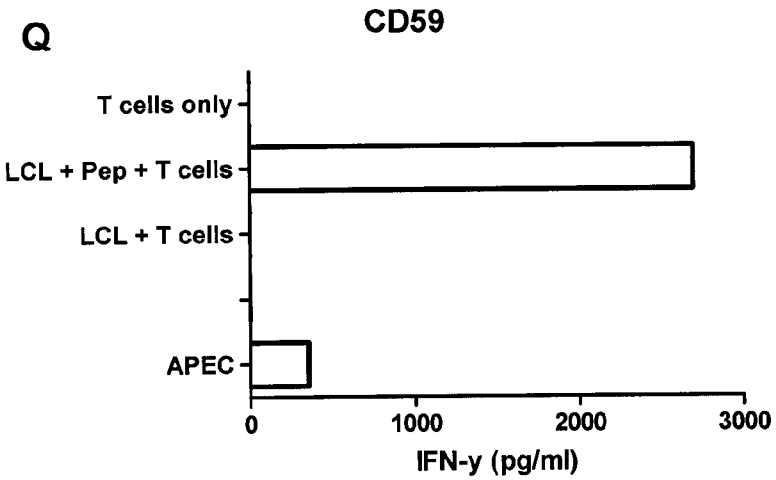

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