



(51) International Patent Classification:

G01N 33/569 (2006.01) A61L 2/00 (2006.01)
G01N 33/50 (2006.01) A61K 45/06 (2006.01)
G01N 33/68 (2006.01)

(21) International Application Number:

PCT/GB2012/051094

(22) International Filing Date:

16 May 2012 (16.05.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1108272.4 17 May 2011 (17.05.2011) GB

(71) Applicant (for all designated States except US): **CAMBRIDGE ENTERPRISE LIMITED** [GB/GB]; The Old Schools, Trinity Lane, Cambridge, Cambridgeshire CB2 1TN (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **LEHNER, Paul** [GB/GB]; Cambridge Institute for Medical Research, University of Cambridge, Wellcome Trust/MRC Building, Addenbrooke's Hospital, Hills Road Cambridge Cambridgeshire CB2 0XY (GB). **SINCLAIR, John** [GB/GB]; Department of Medicine, University of Cambridge, Level 5 Laboratory Block (Box 157), Addenbrooke's Hospital, Hills Road Cambridge Cambridgeshire CB2 2QR (GB). **WEEKS, Michael** [GB/GB]; Cambridge Institute for Medical Research, University of Cambridge, Wellcome Trust/MRC Building, Addenbrooke's Hospital, Hills Road Cambridge Cambridgeshire CB2 0XY (GB). **ANTROBUS, Philip** [GB/GB]; Cambridge Institute for Medical Research, University of Cambridge, Wellcome Trust/MRC

Building, Addenbrooke's Hospital, Hills Road Cambridge Cambridgeshire CB2 0XY (GB).

(74) Agents: **SUTCLIFFE, Nicholas** et al.; Mewburn Ellis LLP, 33 Gutter Lane, London, Greater London EC2V 8AS (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: DETECTION AND DEPLETION OF HCMV INFECTED CELLS

(57) Abstract: This invention relates to the identification and/or elimination of Human Cytomegalovirus (HCMV) infected cells from mammalian cell populations by determining the expression of multidrug resistance-associated protein-1 (MRP-1) and/or other UL138 modulated cell surface proteins. HCMV infected cells may be identified through alterations in the expression MRP-1 and/or other UL138 modulated cell surface proteins, and separated. Mammalian cell populations depleted of HCMV infected cells may be useful, for example in transplants, such as bone marrow transplants.



DETECTION AND DEPLETION OF HCMV INFECTED CELLS

This invention relates to the identification and/or elimination of HCMV-infected cells from populations of mammalian cells.

Human Cytomegalovirus (HCMV; also known as human herpes virus 5 HHV-5) is a ubiquitous beta-herpes virus that infects 60-90% of individuals (1). Following primary infection, HCMV typically establishes a latent infection under the control of a healthy immune system (2). Reactivation from viral latency to productive infection can lead to serious disease in immunocompromised individuals, and is a particular problem in bone marrow transplant recipients where HCMV disease remains one of the most significant causes of morbidity and mortality (1).

Approximately 1,200 allogeneic bone marrow transplants are performed per year in the UK alone; and worldwide at least 20 000 transplants. There is evidence that D(+)R(-) (Donors who are HCMV seropositive, recipients who are HCMV seronegative) bone marrow recipients are at particular risk of death - one study reported one year mortality after transplant of 9.7% D(-)R(-) compared to 18.3% D(+)R(-) (3). This increase in death is likely to relate to primary HCMV infection in previously HCMV negative recipients. Transfusion-mediated HCMV disease can be prevented by leukocyte depletion of blood, suggesting that HCMV is transmitted as latent virus in the peripheral blood leukocyte population. CD34+ myeloid progenitor cells are implicated in latent HCMV carriage in naturally infected individuals (4, 5).

Three viral genes are known to be expressed during natural latency including HCMV UL138 (6). A recent study characterised pUL138 as a golgi-associated protein, though its function remains unknown (7).

The ability to deplete latently infected HCMV-carrying cells would decrease the incidence of HCMV disease in bone marrow transplant recipients, thus decreasing morbidity and mortality. However, to date it has not been possible to identify these rare HCMV infected cells in the bone marrow. The ability to purify latently infected HCMV-carrying cells would be of considerable value to the research community.

The present inventors have identified cell surface proteins whose expression is altered in latent HCMV infection and which can be used to identify and/or eliminate HCMV infected cells from a cell population.

An aspect of the invention provides a method of screening a population of mammalian cells for HCMV infection comprising:

providing a population of mammalian cells and
determining in one or more cells in the population the expression of one or more UL138 modulated cell surface proteins selected from the group consisting of multidrug resistance-associated protein 1 (MRP-1), membrane transport protein XK, delta-like protein 1, platelet glycoprotein 4, tumour necrosis factor receptor superfamily member 1A and neuronal growth regulator 1,

wherein altered expression of the UL138 modulated cell surface proteins relative to controls is indicative that a cell is infected with HCMV.

A UL138 modulated cell surface protein is a protein on the mammalian cell surface whose expression is altered by expression of the HCMV protein UL138.

A method may comprise determining the expression of one or more, two or more, three or more, four or more or five or more UL138 modulated cell surface proteins in one or more cells in the population. Preferably, the cell surface proteins whose expression is determined include MRP-1.

As shown in Table 1, reduced expression of one or more UL138 modulated cell surface proteins selected from the group consisting of multidrug resistance-associated protein 1 (MRP-1), membrane transport protein XK, and delta-like protein 1 relative to controls may be indicative that a cell is infected with HCMV.

As shown in Table 1, increased expression of the one or more UL138 modulated cell surface proteins selected from the group consisting of platelet glycoprotein 4, tumor necrosis factor receptor superfamily

member 1A and neuronal growth regulator 1 relative to controls may be indicative that a cell is infected with HCMV.

In preferred embodiments, a method may comprise determining the expression of MRP-1 and, optionally one or more other UL138 modulated cell surface proteins as described herein.

Methods may be useful in identifying HCMV infected mammalian cells in a population. A method may comprise:

identifying in the population one or more cells with altered UL138 modulated cell surface protein expression relative to controls, wherein said one or more mammalian cells are HCMV infected cells.

In preferred embodiments, a method may comprise identifying in the population one or more cells with reduced expression of MRP-1 and, optionally altered expression of one or more other UL138 modulated cell surface proteins as described herein.

Methods may be useful in identifying non-HCMV infected mammalian cells in a population. A method may comprise:

identifying in the population one or more cells in which UL138 modulated cell surface protein expression is not altered relative to controls,

wherein said one or more mammalian cells are not infected with HCMV.

In preferred embodiments, a method may comprise identifying in the population one or more cells in which the expression of MRP-1 and, optionally one or more other UL138 modulated cell surface proteins as described herein, is not altered.

In preferred embodiments, the expression of the UL138 modulated cell surface protein MRP-1 is determined. Optionally, the expression of one or more other UL138 modulated cell surface proteins in addition to MRP-1 may also be determined.

A method of screening a population of mammalian cells for HCMV infection may comprise:

- providing a population of mammalian cells and
- determining the expression of MRP-1 in one or more cells in the population,
- wherein reduced MRP-1 expression relative to controls is indicative that a cell is infected with HCMV.

As described above, methods may be useful in identifying HCMV infected mammalian cells in a population. A method may comprise:

- identifying in the population one or more cells with reduced MRP-1 expression relative to controls,
- wherein said one or more mammalian cells are HCMV infected cells.

Methods may also be useful in identifying non-HCMV infected mammalian cells in a population. A method may comprise:

- identifying in the population one or more cells in which MRP-1 expression is not reduced relative to controls,
- wherein said one or more mammalian cells are not infected with HCMV.

Suitable control cells include non-HCMV infected mammalian cells of the same type as those in the population.

MRP-1 (Multidrug resistance-associated protein 1; also known as ABCC1; Gene ID 4363) is a multi-specific organic anion transporter which is involved in multidrug resistance. Reference amino acid sequences of MRP1 are available on public databases and include NP_004987.2 GI: 134142337. Reference amino acid sequences of other UL138 modulated cell surface protein shown in Table 1 are also available on public databases, as shown in Table 2.

A cell with altered expression of a UL138 modulated cell surface protein displays reduced amounts of the UL138 modulated protein on its surface i.e. reduced amounts of the UL138 modulated protein are present in the plasma membrane of the cell. For example, a cell with reduced MRP-1

expression displays reduced amounts of MRP-1 on its surface i.e. reduced amounts of MRP-1 are present in the plasma membrane of the cell.

The expression of a UL138 modulated cell surface protein may be increased or reduced by 50% or more, 60% or more, 70% or more, 80% or more, or 85% or more in HCMV infected cells relative to controls (i.e. identical cells which are not infected with HCMV). For example, UL138 modulated cell surface protein expression may be up- or down- regulated by 2 fold or more, 3 fold or more, 4-fold or more, 5-fold or more, 6-fold or more or 7-fold or more in an HCMV infected cell relative to a non-HCMV infected cell.

For example, MRP-1 expression may be reduced by 50% or more, 60% or more, 70% or more, 80% or more, or 85% or more in HCMV infected cells relative to controls (i.e. identical cells which are not infected with HCMV). For example, MRP-1 expression may be down regulated by 2 fold or more, 3 fold or more, 4-fold or more, 5-fold or more, 6-fold or more or 7-fold or more in an HCMV infected cell relative to a non-HCMV infected cell.

HCMV infected mammalian cells may be latently infected with HCMV. Mammalian cells with latent HCMV infection may contain dormant HCMV virus which is not replicating or producing new virus but may be reactivated at a later date. UL138 is expressed by HCMV virus in latently infected mammalian cells.

The mammalian cells are preferably human cells.

The population of mammalian cells may be isolated and may be obtained from an isolated or cultured cell line or may be obtained from a tissue or sample of tissue.

Suitable tissues include haematopoietic tissues such as bone marrow. For example, a method of screening bone marrow cells for HCMV infection as described herein may comprise:

providing a population of bone marrow cells and

determining the expression of one or more UL138 modulated cell surface proteins in one or more cells in the population,

wherein altered UL138 modulated cell surface protein expression relative to controls is indicative that a bone marrow cell is infected with HCMV.

In some embodiments, the UL138 modulated cell surface proteins may include MRP-1. Reduced MRP-1 expression relative to controls is indicative that a bone marrow cell is infected with HCMV.

A population of cells, for example bone marrow cells, may be obtained or isolated from a sample obtained from a donor individual. The provision of tissue samples from a donor individual is well-known in the art.

Cells which express altered amounts of a UL138 modulated cell surface protein, such as MRP-1, may be identified using a suitable binding member. For example, expression of a UL138 modulated cell surface protein may be determined by;

(i) contacting the population of mammalian cells with a specific binding member which specifically binds to the UL138 modulated cell surface protein on a cell surface and

(ii) determining the binding of the binding member to the cells in the population,

wherein a difference, for example an increase or decrease, in binding to a cell relative to controls is indicative that the cell has altered UL138 modulated cell surface protein expression.

One or more cells in the population to which the binding member shows altered binding relative to controls may be identified. Altered binding is indication of altered expression of a UL138 modulated cell surface protein.

One or more cells with altered, for example reduced, UL138 modulated cell surface protein expression relative to controls may be identified in the population, these one or more mammalian cells being HCMV infected.

UL138 modulated cell surface proteins for which increases or decreases in expression are indicative of HCMV infection are described above.

Preferably, the UL138 modulated cell surface protein is MRP-1. Reduced binding of the binding member is indication of reduced expression of MRP-1.

One or more cells in the population to which the binding member does not show altered binding may be identified. The absence of altered binding is indicative of normal unaltered expression of a UL138 modulated cell surface protein.

One or more cells in which UL138 modulated cell surface protein expression is not altered relative to controls may be identified in the population, these one or more mammalian cells being non-HCMV infected

Suitable specific binding members bind to cells in the population which express an UL138 modulated protein, such as MRP-1, on the cell surface and do not bind to cells which do not express the UL138 modulated protein. For example, a suitable binding member may bind to an extracellular domain of a UL138 modulated protein.

Specific binding members may include antibodies and antibody fragments and derivatives. The production of suitable antibody molecules is described below.

A population of mammalian cells may contain a sub-population of cells infected with HCMV and a sub-population of cells not infected with HCMV. Methods of the invention may be useful in enriching, depleting or eliminating one of these sub-populations.

Typically, the proportion of cells in a mammalian cell population which are infected with HCMV is less than 1%.

Cells which express altered amounts of a UL138 modulated cell surface protein, such as reduced amounts of MRP-1, may be identified by any convenient technique. For example, the amount of UL138 modulated cell surface protein which is present at the cell surface may be determined

either directly or using functional assays. Preferred techniques for cell identification are non-destructive and do not significantly reduce the viability of the cells in the population. Following identification the cells may be isolated, as described below

In preferred embodiments of the invention, the UL138 modulated cell surface protein is MRP-1.

Mammalian cells which express reduced amounts of MRP-1 may be identified by reporter-based techniques. For example, a method may comprise:

- (i) incubating the population of mammalian cells with a reporter molecule which is selectively expelled from cells by MRP-1,
- (ii) identifying one or more cells in the population in which the reporter molecule accumulates, wherein said one or more cells are HCMV infected, and/or;
- (iii) identifying one or more cells in the population in which the reporter molecule does not accumulate, wherein said one or more cells are not HCMV infected.

A reporter molecule is a molecule which is capable of producing a detectable signal. For example, a reporter molecule may produce a fluorescent or chromogenic signal which can be detected using conventional techniques. Suitable reporter molecules for use as described herein are selectively expelled from mammalian cells by MRP-1 i.e. reporter molecules are actively expelled from the interior of mammalian cell by MRP-1 but are not expelled by other cell membrane drug transporters, such as p-glycoprotein.

Suitable dyes include fluorescent dyes, such as seminaphtharhodafluor (SNARF-1). Other suitable fluorescent dyes are known in the art.

Cells which express reduced amounts of MRP-1 may be identified using a suitable binding member. For example, a method may comprise:

- (i) contacting a population of mammalian cells with a binding member which specifically binds to MRP-1 on a cell surface,

(ii) identifying one or more cells in the population which bind to the binding member, wherein said one or more cells are non-HCMV infected cells, and/or;

(iii) identifying one or more cells in the population which do not bind to the binding member, wherein said one or more cells are HCMV infected cells.

Suitable binding members bind to cells in the population which express MRP-1 on the cell surface and do not bind to cells which do not express MRP-1. For example, a suitable binding member may bind to an extracellular domain of MRP-1.

Suitable binding members include antibodies, antibody fragments and derivatives, including scFvs and nanobodies, DARPs, affibodies and anticalins.

Antibodies may include monoclonal or polyclonal antibodies.

The production of antibodies is standard in the art. Methods of producing antibodies include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to antigen of interest. For instance, Flow cytometry, Western blotting techniques or immunoprecipitation may be used (Armitage et al., 1992, Nature 357: 80-82).

As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a protein may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with any of the proteins (or fragments), or may be one constructed using sequences obtained from an organism which has been exposed to the antigen of interest.

In some embodiments, a binding member, such as an antibody, which specifically binds to MRP-1 on a cell surface may be labelled with a detectable label.

Suitable detectable labels are well known in the art and include fluorescent labels, such as FITC or rhodamine, chromogenic labels, epitope tags and non-isotopic labelling reagents, such as biotin or digoxigenin.

A labelled antibody may be contacted with the population of cells and binding to one or more cells in the population detected by measuring the presence of detectable label at the cell surface.

In other embodiments, a secondary antibody labelled with a detectable label may be used to detect binding. For example, a first antibody which specifically binds to MRP-1 on a cell surface may be contacted with the population of cells and then the population may be contacted with a second antibody which binds to the first antibody and is labelled with a detectable label. The presence of detectable label at the surface of one or more cells in the population may then be determined.

Cells in the population identified as having reduced MRP-1 expression, for example through increased reporter molecule accumulation or reduced binding of an MRP-1 specific binding member may be identified as HCMV infected, as described herein.

Cells in the population identified as not having reduced MRP-1 expression, for example through the absence of any increase in reporter molecule accumulation or reduction in binding of an MRP-1 specific binding member may be identified as non-HCMV infected, as described herein.

One or more cells in a population may be identified as HCMV infected or non-HCMV infected using a method described above. Following identification, the identified cells may be separated from the other cells in the population. The depletion or enrichment of HCMV infected or

uninfected cells in a mammalian cell population is described in more detail below.

Any convenient technique may be employed to separate cells identified as HCMV infected or non-HCMV infected. For example, the cells may be separated by flow cytometry, for example fluorescence activated cell sorting (FACS). Suitable flow cytometry techniques are well known in the art.

HCMV infected or non-infected cells removed from the population may be retained, stored and/or cultured for further research or investigation.

Other aspects of the invention relate to the removal or depletion of cells which are infected with HCMV or cells which are not infected with HCMV from a population of mammalian cells. This may be useful for example, in providing a population of non-HCMV infected cells for therapeutic use. Suitable methods may include *ex vivo* and *in vitro* methods.

In some embodiments, HCMV infected and non-infected cells may be separated to produce a population of mammalian cells which is depleted of HCMV infected cells i.e. HCMV infected cells may be eliminated or removed from the population such that population of non-HCMV infected cells is enriched or purified. For example, the purified population may consist predominantly or wholly of non-HCMV infected cells. This population may be useful for transplantation into a recipient individual.

In other embodiments, HCMV infected and non-infected cells may be separated to produce a population of mammalian cells which is enriched for HCMV infected cells i.e. cells not infected with HCMV may be eliminated or removed from the population such that population of HCMV infected cells is enriched or purified. For example, the purified population may consist predominantly or wholly of HCMV infected cells. This population may be useful for research purposes, e.g. further investigation of HCMV infection.

Multiple cycles of identification and separation of HCMV non-infected or infected cells as described above may be performed to further enrich the population of HCMV infected or non-HCMV infected cells. For example, one, two, three or more cycles of identification and separation of HCMV infected cells may be performed to produce a pure or essentially pure population of non-HCMV infected mammalian cells. For example, a population of at least 99.9% non-HCMV infected mammalian cells may be produced.

A method of removing HCMV infected cells or non- HCMV infected cells from a population of mammalian cells may comprise;

- (i) providing a population of mammalian cells
- (ii) identifying one or more HCMV infected cells or non-HCMV infected cells in the population using a method described above, and;
- (iii) removing the identified cells from the population and, optionally,
- (iv) repeating steps (ii) and (iii) one or more times.

HCMV infected cells or non-HCMV infected cells in the population may be identified using a method described above.

In some embodiments, a method may comprise;

identifying one or more cells in the population with altered expression of a UL138 modulated cell surface protein relative to controls,

removing the identified cells from the population, and;
optionally repeating these two steps one or more times.

For example, a method may comprise;

identifying one or more cells in the population with reduced expression of MRP-1 relative to controls,

removing the identified cells from the population, and;
optionally repeating these two steps one or more times.

Cells in the population with altered amounts of a UL138 modulated protein on the cell surface, for example reduced amounts of MRP-1, may be identified as HCMV infected cells using a method described above.

Following removal of the HCMV infected cells, the remaining cell population is depleted of HCMV infected cells, preferably completely or substantially devoid of HCMV infected cells.

In other embodiments, a method may comprise;

- identifying one or more cells in the population which do not have altered expression of a UL138 modulated cell surface protein relative to controls,

- removing the identified cells from the population, and;
- optionally repeating these steps one or more times.

For example, a method may comprise;

- identifying one or more cells in the population which do not have reduced expression of MRP-1 relative to controls,

- removing the identified cells from the population, and;
- optionally repeating these steps one or more times.

Cells in the population which do not display altered expression of a UL138 modulated protein, for example reduced amounts of MRP-1, on the cell surface (i.e. normal levels of expression) are non-HCMV infected cells and may be identified using a method described above.

The cells removed from the population form a cohort of cells which is depleted of HCMV infected cells, preferably devoid or substantially devoid of HCMV infected cells.

In some embodiments, a population of mammalian cells may be treated to eliminate or reduce the number of cells in the population which are HCMV infected. For example, a method of depleting HCMV infected cells from a population of mammalian cells may comprise:

- providing a population of mammalian cells, and;
- selectively inducing the death of cells within the population which display reduced amounts of MRP-1 on the cell surface relative to controls,

- wherein said one or more cells are HCMV infected cells.

Cell death may, for example, be selectively induced in HCMV infected cells by a method comprising;

incubating the population of cells with non-toxic levels of a cytotoxic drug which is selectively expelled from cells by MRP-1, wherein said cytotoxic drug accumulates to toxic levels in HCMV infected cells.

The accumulation of toxic levels of the cytotoxic drug causes cell death in HCMV infected cells. The cytotoxic drug is expelled from non-HCMV infected cells by MRP-1, so it does not accumulate to toxic levels. The population of surviving cells is depleted of HCMV infected cells.

Cytotoxic drugs which are selectively expelled from cells by MRP-1 include conventional natural product drugs (e.g., the *Vinca* alkaloids, such as vinblastine and vincristine; anthracyclines, such as doxorubicin and daunorubicin; alkylating agents such as melphalan; platinum-containing compounds, such as cisplatin and carboplatin; antimetabolites, such as methotrexate; topoisomerase inhibitors, such as etoposide, irinotecan and mitoxantrone; mitotic inhibitors, such as paclitaxel; anti-androgens, such as flutamide, and hydroxyflutamide and nucleoside/nucleotide analogues, such as gemcitabine and cytosine arabinoside.

In some embodiments, suitable cytotoxic drugs include doxorubicin and vincristine.

Another aspect of the invention provides a kit for enriching or depleting a cell population for cells infected with HCMV or cells not infected with HCMV comprising:

- (i) one or more solid substrates conjugated with antibodies or other binding members which specifically bind to one or more cell surface proteins whose expression is increased by HCMV infection, and/or
- (ii) one or more solid substrates conjugated with antibodies or other binding members which specifically bind to one or more cell surface proteins whose expression is reduced by HCMV infection, such as MRP-1.

Suitable solid substrates include magnetic beads and cell sorting by flow cytometry.

Suitable cell surface proteins are shown in table 1. For example, cell surface proteins whose expression on the cell surface is increased by HCMV infection may include platelet glycoprotein 4, tumor necrosis factor receptor superfamily member 1A and neuronal growth regulator 1.

Cell surface proteins whose expression on the cell surface is reduced by HCMV infection may include multidrug resistance-associated protein 1 (MRP-1), membrane transport protein XK and delta-like protein 1.

Preferably, the kit comprises wherein one or more solid substrates conjugated with antibodies which specifically bind to MRP-1.

A kit may be used or adapted for use in a method described herein.

Solid substrates and other reagents may be provided in suitable containers, such as vials in which the contents are protected from the external environment. The kit may include instructions for use, e.g. in a method described herein.

Another aspect of the invention provides a method of enriching or depleting a cell population for cells infected with HCMV or cells not infected with HCMV comprising

- (i) contacting the population with one or more immobilised antibodies or other binding members which bind to a cell surface marker whose expression is altered by HCMV infection, such as MRP-1, and;
- (ii) separating bound cells from the population.

Steps (i) and (ii) may be repeated one or more times.

The antibodies or other binding members may bind to a cell surface protein whose expression on the cell surface is reduced by HCMV infection, such as MRP-1. Suitable cell surface proteins are listed above and shown in table 1. A population of cells which bind to the one

or more immobilised antibodies or other binding members may be isolated. The population of cells which bind to the one or more immobilised antibodies may be depleted of cells infected with HCMV. A population of cells which do not bind to the one or more immobilised antibodies or other binding members may be isolated. The population of cells which do not bind to the one or more immobilised antibodies or other binding members may be enriched for cells infected with HCMV.

The antibodies or other binding members may bind to a marker whose expression on the cell surface is increased by HCMV infection. Suitable cell surface proteins are listed above and shown in table 1. A population of cells which bind to the one or more immobilised antibodies or other binding members may be isolated. The population of cells which bind to the one or more immobilised antibodies or other binding members may be enriched for cells infected with HCMV. A sub-population of cells which do not bind to the one or more immobilised antibodies or other binding members may be isolated. The sub-population of cells which do not bind to the one or more immobilised antibodies or other binding members may be depleted of cells infected with HCMV.

A population of mammalian cells which has been treated as described herein to remove or eliminate cells which are infected with HCMV may be useful as a medicament, for example in transplantation.

Other aspects of the invention provide a population of mammalian cells depleted of HCMV infected cells using a method described herein; a population of mammalian cells depleted of HCMV infected cells using a method described herein for use as a medicament, for example in the replacement of dysfunctional or damaged tissue in an individual; and the use of a population of mammalian cells depleted of HCMV infected cells using a method described herein in the manufacture of a medicament for use in the replacement of dysfunctional or damaged tissue in an individual.

A method of treating a patient with a damaged or dysfunctional tissue may comprise;

administering a population of mammalian cells depleted of HCMV infected cells as described above to an individual in need thereof.

Individuals suitable for treatment include HCMV negative individuals.

Suitable mammalian cell populations include samples of bone marrow. A sample of bone marrow depleted of HCMV infected cells using a method described herein may be used as a medicament, for example for transplantation into a recipient individual in need thereof; and the use of sample of bone marrow depleted of HCMV infected cells using a method described herein in the manufacture of a medicament for transplantation into a recipient individual in need thereof.

A suitable individual may have diseased, damaged or dysfunctional bone marrow.

In some embodiments, the recipient individual may be HCMV negative. An individual may be identified as HCMV negative using standard techniques.

The invention also extends to a pharmaceutical composition, medicament, drug or other composition comprising a population of mammalian cells depleted of HCMV infected cells using a method described herein, a method comprising administration of such a population of mammalian cells depleted of HCMV infected cells to a patient, e.g. for treatment (which may include preventative treatment) of damaged or dysfunctional tissue, as described above, and a method of making a pharmaceutical composition comprising admixing such a population of mammalian cells depleted of HCMV infected cells with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally one or more other ingredients.

A composition may contain a population of mammalian cells depleted of HCMV infected cells using a method described herein, and one or more additional components. Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to the mammalian cells, a pharmaceutically acceptable excipient, carrier, buffer, preservative, stabiliser, anti-oxidant or other material well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the activity

of the mammalian cells. The precise nature of the carrier or other material will depend on the route of administration.

Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, tissue or cell culture media, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

The composition may be in the form of a parenterally acceptable aqueous solution, which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride, Ringer's Injection, or Lactated Ringer's Injection. A composition may be prepared using artificial cerebrospinal fluid.

Mammalian cells may be implanted into a patient by any technique known in the art (e.g. Lindvall, O. (1998) *Mov. Disord.* 13, Suppl. 1:83-7; Freed, C.R., et al., (1997) *Cell Transplant*, 6, 201-202; Kordower, et al., (1995) *New England Journal of Medicine*, 332, 1118-1124; Freed, C.R., (1992) *New England Journal of Medicine*, 327, 1549-1555, Le Blanc et al, *Lancet* 2004 May 1;363(9419):1439-41). In particular cell suspensions may be injected into a suitable vein of a patient.

Administration of a composition in accordance with the present invention is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Other aspects of the invention relate to screening methods for agents useful in the methods described herein.

A method of screening for an agent which is useful in depleting or eliminating HCMV infected cells in a population may comprise;

determining the accumulation of a test compound in an MRP-1 deficient cell relative to a non-MRP-1 deficient cell,

wherein increased accumulation in the MRP-1 deficient cells relative to the non-MRP-1 deficient cell is indicative that the compound is useful in identifying, depleting or eliminating HCMV infected cells in a population.

MRP-1 deficient cells are cells which are deficient in MRP-1 activity. For example, the MRP-1 gene may be inactivated in the cells, expression of the MRP-1 gene may be suppressed, for example by HCMV infection or by RNAi, or the activity of MRP-1 may be inhibited by an inhibitor, such as MK-571 or reversan.

In some embodiments, the test compound may be a cytotoxic agent.

The accumulation of the compound in the MRP-1 deficient cells relative to control cells may be determined by measuring the amount of cell death in the cells. An increase in cell death in MRP-1 deficient cells relative to control cells is indicative that the compound accumulates in MRP-1 deficient cells and may be useful in depleting or eliminating HCMV infected cells in a population. For example, a method of screening for an agent which is useful in depleting or eliminating HCMV infected cells from a population may comprise;

determining the effect of a test compound on a population of MRP-1 deficient cells relative to controls,

wherein an increase in cell death in the MRP-1 deficient population relative to controls is indicative that the compound is useful in depleting or eliminating HCMV infected cells from a mammalian cell population.

In other embodiments, the test compound may be a reporter molecule, such as a fluorescent dye, which produces a detectable signal.

The accumulation of the compound in the MRP-1 deficient cells relative to control cells may be determined by measuring the amount of reporter molecule in the cells for example by measuring the detectable signal produced. An increase in the amount of reporter molecule in MRP-1 deficient cells relative to control cells is indicative that the compound accumulates in MRP-1 deficient cells and may be useful in identifying HCMV infected cells. A method of screening for an agent which is useful in identifying HCMV infected cells from a tissue comprising;

determining the accumulation of a dye in a population of MRP-1 deficient cells relative to controls,

wherein an increase in accumulation in the MRP-1 deficient population relative to controls is indicative that the compound is useful in identifying HCMV infected cells as compared with non-HCMV infected cells in a population.

Compounds identified using the above methods may be useful in the methods described herein.

Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

All documents mentioned in this specification, including sequence database entries, are incorporated herein by reference in their entirety.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or

embodiment of the invention and apply equally to all aspects and embodiments which are described.

Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the figures described below.

Figure 1 shows the analysis of cell surface protein expression in THP-UL138 cells (light) and control cells (dark) by flow cytometry (FACS). The fold changes as determined by PMP (upper number) and FACS (lower number) are shown.

Figure 2 shows immunoblots which confirm that MRP-1 is down-regulated in THP-UL138 cells.

Figure 3 shows immunoblots which confirm that MRP-1 is down-regulated in cells infected with HCMV.

Figure 4 shows the flow cytometry (FACS) analysis THP-UL138 cells (dark) and control cells (light) following loading with fluorescent dye.

Figure 5 shows the down-regulation and loss of cell surface MRP1 expression in latent HCMV infected cells.

Figure 6 shows levels of UL138 as determined by qRT-PCR in latently infected monocytes at 8 days following vincristine treatment.

Table 1 shows the fold down-(1a) or up-(1b) regulation of cell surface proteins by HCMV UL138.

Table 2 shows database references for UL138 modulated proteins.

Experiments

Materials and Methods

Cell culture

Sultan, THP-1 and Jurkat cells were grown in RPMI 1640 medium (Thermo Pierce, Cramlington, UK) supplemented with 10% Foetal Calf Serum (FCS)

(PAA, Yeovil, UK), Penicillin at 100 units / ml and Streptomycin at 0.1mg/ml (Sigma, Dorset, UK).

Preparation of PM proteins using aminooxy-biotin.

Cells were biotinylated, and plasma membrane proteins prepared as described (8). Briefly, cells were washed twice in 50 ml ice-cold PBS pH 7.4 with CaCl₂ and MgCl₂ (Sigma). Surface sialic acid residues were oxidised with 1 mM sodium meta-periodate for 20 min in the dark at 4°C. The oxidation mixture was quenched by addition of glycerol to a final concentration 1 mM. Cells were washed twice in PBS pH 7.4 / 5% (v/v) FCS, then biotinylated in 100 mM aminooxy-biotin (Biotium Inc, Hayward, CA) and 10 mM aniline (Sigma) in PBS pH 6.7 / 5% (v/v) FCS. After 1 h at 4°C, cells were washed once with PBS pH 7.4 / 5% (v/v) FCS then once with PBS pH 7.4. Biotinylated cells were spun at 400 g at 4°C, and the resulting cell pellet was re-suspended and incubated at 4°C for 30 min in lysis buffer (1% Triton X-100 (high purity, Thermo), 150 mM NaCl, 1x protease inhibitor (complete, without EDTA (Roche)), 5 mM iodoacetamide (Sigma), 0.1 mg/ml PMSF and 10 mM Tris-HCl pH 7.6). Nuclei were removed by centrifugation at 4°C three times for 10 min at 2,800g then 16,000g. Biotinylated proteins were enriched by incubating for 2 h at 4°C with high affinity streptavidin agarose (Pierce). Extensive washing was performed using a vacuum manifold and Snap Cap spin columns (Pierce), with intermittent centrifugation at 1,000g for 1 min to ensure complete removal of wash buffers. Beads were initially washed with lysis buffer, then PBS / 0.5% (w/v) SDS. Beads were next incubated for 20 min at RT with PBS / 0.5% (w/v) SDS / 100 mM DTT. Further washing was performed using UC buffer (6 M urea, 100 mM Tris-HCl pH 8.5), followed by incubation for 20 min at RT with UC buffer containing 50 mM iodoacetamide. Beads were washed with UC buffer, then PBS, then water, and biotinylated glycoproteins were digested on-bead overnight in 400µl 50mM NH₄HCO₃ containing 4 µg modified sequencing grade trypsin (Promega). Tryptic peptides were collected by centrifugation at 1,000g for 1 min. Beads were washed once with 50mM NH₄HCO₃, and pooled tryptic fractions were either desalted using StageTips (9), or fractionated by SAX or HPLC (see below). To elute glycopeptides, beads were washed with PBS, then water, then G7 buffer (New England Biolabs, Hitchin, UK). Beads were incubated overnight in 400 µl G7 buffer containing 30,000

units of glycerol free PNGase (New England Biolabs). Glycopeptides were collected by centrifugation at 1,000g for 1 min, beads were washed once with G7 buffer, and eluates pooled and desalted using StageTips (9).

Strong Anion Exchange (SAX) fractionation and mass spectrometric analysis

SAX was performed as described by Wisniewski et al (10). Briefly, 30 - 50 µg of tryptic peptide was loaded at pH 11 on a tip-based anion exchanger constructed using 6 layers of Empore anion exchange disk (3M, Bracknell, UK). The column was equilibrated and fractions eluted using Britton & Robinson buffer (20 mM acetic acid, 20 mM phosphoric acid, 20 mM boric acid titrated with NaOH to the desired pH). Fractions were subsequently eluted with buffer solutions of pH 11, 8, 6, 5, 4, and 3 onto StageTips (9) containing three layers of C18 membrane.

Fractionated samples were eluted from StageTips, dried almost to completion using an Eppendorf concentrator and re-suspended in 11 µl MS solvent (3% (v/v) MeCN, 0.1% (v/v) formic acid). For each LC-MSMS run 3 µl was injected onto a NanoAcquity uPLC (Waters, Milford, MA, USA). Peptides were loaded onto a 180 µm / 20 mm Symmetry C18 trap column (Waters) for 1 min at 15 µl / min and eluted to a 75 µm / 150 mm BEH130 C18 analytical column. Peptides were eluted with a MeCN gradient rising from 3 % to 25 % by 130 min, 25 % to 40 % by 205 min and to 85 % by 210 min. The total run time was 240 min. Peptides were eluted into the LTQ OrbiTrap XL (Thermo) via 10 µm SilicaTip emitters (New Objectives, MA, USA). Duplicate gradients were performed for each fraction. Data was acquired by a Top 10 data dependent acquisition (DDA) method with survey scans acquired at 60,000 resolution (FWHM at m/z 400).

High pressure liquid chromatography (HPLC) fractionation

A total of 144 µg of tryptic peptides were subjected to high pH reverse-phase (RP) fractionation using a Dionex ICS-3000 with an Agilent ZORBAX extend column (4.6 mm x 150 mm). Mobile phase buffer A was H₂O, 0.1 % NH₃ pH 10.5 and buffer B MeCN, 0.1 % NH₃. Peptides were resolved using a gradient 0.5 - 40 % B over 48 mins at at a 400 µl/min flow rate and a column temperature of 15°C. Eluting peptides were collected in 15s fractions.

Fractions were dried down using an Eppendorf Concentrator and re-suspended in 8 µl MS solvent (3 % MeCN, 0.1 % TFA). Fractions 25 to 130 inclusive were analysed and in each case 3 µl was injected and subjected to LC-MSMS using a NanoAcquity uPLC coupled to an LTQ-Orbitrap XL. Peptides were eluted using a gradient rising to 25 % MeCN by 33 min and 40 % MeCN by 36 min. The column was washed at 85 % MeCN and re-equilibrated at 3 % MeCN for 30 min. MS data was acquired at 60,000 (FWHM at 400 m/z) with lockmass enabled (445.120025 m/z). CID spectra were acquired in the LTQ with MS/MS switching operating in a top 6 DDA fashion triggering at 500 counts.

Database searching and data processing

Raw MS files were processed using MaxQuant versions 1.0.12.31 or 1.0.13.13(11) with .msm output files searched against decoy International Protein Index human database version 3.68 (12) containing both forward and reverse protein sequences using MASCOT Daemon 2.2.0 (13). Fragment ion tolerance was set to 0.5 Da with a maximum of 2 missed tryptic cleavage sites. Carbamidomethyl cysteine was defined as a fixed modification, oxidised methionine, deamidation (NQ) and in the NHS-SS-biotin experiments CAMthiopropionyl (K and protein N-terminus) were selected as variable modifications. The false discovery rate for both peptides and proteins were set at 0.01. Gene Ontology (GO) information was annotated to data by MaxQuant (11). Protein quantitation utilised razor and unique peptides and required a minimum of 2 ratio counts. Peptide requantitation was enabled.

Generation of UL138-expressing THP-1 cell lines

Full-length UL138 expressed in pcDNA3 was completely digested with NotI then subjected to partial digest with BamHI (37°C, 10 minutes). DNA fragments were separated on a 2% agarose gel, and bands visualised briefly with Ultraviolet light. The band corresponding to full-length UL138 (as opposed to that cut internally with BamHI) was excised, and DNA purified. UL138 was cloned into a lentiviral expression plasmid (pHRSin-UbEmerald). The insert was checked by DNA sequencing, and stable THP-1 cells expressing UL138 generated by lentiviral transduction. Cells expressing a high level of UL138 (Emerald high) were sorted on a FACS

Vantage. For the fluorescent dye assay, a separate THP-UL138 cell line was generated using a second lentiviral vector without Emerald.

Flow cytometry

Cells were washed in PBSBSA (0.1%), incubated (15 min, 4°C) with 10% rabbit serum, and stained (30min, 4°C) using the indicated monoclonal antibody. Antibodies were sourced from: unconjugated anti-TNFR1 (16803, R and D systems), PE-conjugated anti-DLL1 (MHD1-314, BioLegend). Cells were washed in PBSBSA (0.1%), and stained using APC-conjugated Streptavidin (eBioscience) (for biotinylated antibodies), or goat anti-mouse Cy5 (Jackson ImmunoResearch) for 30 min at 4°C. APC is handled orthogonally to FITC by the FACS Calibur, thus there is no interference from cells stained using a green or red-conjugated antibody. Cells were bright green due to Emerald expression, hence needed assessment with a differently-conjugated antibody. After one wash in ice-cold PBS, cells were fixed in 4% paraformaldehyde and analysed on a FACS Calibur using Cellquest (BD Pharmingen) and FCSPress v1.3 (www.fcspress.com).

Immunoblotting

Cells were lysed in 1% Triton X-100, in TBS pH8 with 0.5 mM PMSF, 10 mM iodoacetamide, and Roche complete protease inhibitor for 30 min on ice. Postnuclear supernatants were heated at 50°C in SDS sample buffer, separated by SDS/PAGE, and proteins transferred to PVDF membranes (Millipore). Membranes were probed with the indicated antibodies (anti-human MRP1 antibody, QCRL-1, R and D systems; anti-Calnexin, anti- β -actin (Sigma)). We generated a new anti-UL138 rabbit polyclonal antibody (Cambridge Research Biochemicals). Reactive bands were visualized with West Pico or West Dura (ThermoFisher Scientific).

Infection of fibroblasts with HCMV

A low-passage laboratory HCMV strain was purified from infected fibroblasts on sorbitol gradients as described previously (14). Human fetal foreskin fibroblasts were maintained in minimal essential medium (Gibco-BRL) containing 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 mg/ml). Cells were infected at an MOI of (5:1) for 24 hours prior to harvest for immunoblot.

Fluorescent dye assay

Seminaphtharhodafleur or SNARF-1 is a fluorescent dye that is pumped out of cells by MRP-1. THP-1 and fibroblasts expressing UL138, fibroblasts infected with HCMV or control cells were loaded for one hour with SNARF-1 (3 - 5uM) and then washed. Dye retention over time (up to 8 hours) was measured using flow cytometry. The MRP1-specific inhibitor MK-571 was used as a control for MRP1 specific efflux.

Results

'Plasma membrane profiling' (PMP) was developed to identify novel cell surface proteins whose expression is altered upon infection with any intracellular pathogen. Using this unbiased approach we examined how viral infection alters the expression of more than 600 plasma membrane proteins (8). We have used PMP to identify which plasma membrane proteins are affected by HCMV latency protein UL138.

We generated a UL138-expressing monocytic cell line (THP-UL138). We used PMP to compare levels of cell surface protein expression in a THP-UL138 and control cells. We performed three independent experiments - the fold down- or up- regulation of each protein is shown in Table 1 for each experiment performed.

Significant changes were identified in 20/606 proteins (Table 1a, b).

Of the cell surface proteins affected by UL138, the most markedly down-regulated is the drug transporter Multiple Drug Resistance Protein 1 (MRP-1). MRP-1 is a multi-specific transporter that exports certain cytotoxic drugs and fluorescent dyes from the cell. A variety of other cell surface proteins are also up- or down-regulated, including for example signal regulatory protein beta (SIRPB1) whose expression is reduced 3-3.5 fold in THP-1 cells expressing UL138.

We used flow cytometry with commercially available antibodies to confirm down- or up-regulation of a number of UL138 targets (Figure 1).

Our observation of MRP-1 down-regulation was confirmed using commercial antibodies that recognise MRP-1 by immunoblot. THP-UL138 cells were

grown in specialist growth media used for PMP as well as in normal media (Figure 2). Furthermore, the functional relevance of these observations was confirmed in HCMV-infected cells (Figure 3).

Both control cells and UL138-expressing cells were loaded for one hour with the MRP-1 specific fluorescent dye seminaphtharhodafluor (SNARF-1) and dye retention over time was measured. HCMV-infected cells were found to accumulate fluorescent dye and were unable to efficiently remove it. At 3 hours, the control and UL138-expressing populations are easily distinguishable (Figure 4). Down-regulation of MRP-1 is therefore associated with dye retention.

In an experimental model of HCMV latent phase infection in human monocytic cell lines, latent HCMV infected cells were found to show a marked down regulation and loss of cell surface MRP1 expression (Figure 5). These results confirm that, in latent phase HCMV infection, UL138 expression down regulates MRP1 expression.

In an experimental model of HCMV latent phase infection in human monocytic cell lines, treatment of cells with an increasing dose of vincristine (a cytotoxic drug and specific substrate of MRP1) was found to preferentially kill latently infected cells, as determined by a decrease in latently infected cells, and a concomitant decrease in detectable UL138 by quantitative rtPCR (Figure 6). The loss of latently infected cells is due to an accumulation of the drug within infected cells which are unable to excrete vincristine in the absence of MRP1 activity.

These results demonstrate that the MRP1 drug transporter is down regulated in latently infected HCMV cells, and also that this MRP1 down regulation may be used for therapeutic advantage by killing infected cells which are unable to pump out cytotoxic MRP-1 specific substrates, such as vincristine.

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Protein Names	Gene name	Fold downregulation		
		Expt 3	Expt 2	Expt 1
Multidrug resistance-associated protein 1	ABCC1	7.1	8.8	6.1
MHC class II antigen	HLA-DRB1	4.6	5.7	4.7
Membrane transport protein XK	XK	5.3	5.6	
HLA class II histocompatibility antigen, DR alpha chain	HLA-DRA	5.2	5.4	
Syntaxin-4	STX4	4.6		
Vacuolar protein sorting-associated protein 13A	VPS13A	4.2		
SLAM family member 5	CD84	3.1		4.2
Signal-regulatory protein beta-1	SIRPB1	3.0	3.0	3.5
C-X-C chemokine receptor type 3	CXCR3	1.6	2.8	
Putative uncharacterized protein C10orf128	C10orf128		2.8	
Cytochrome b-245 heavy chain	CYBB	2.5	2.6	3.1
Seizure 6-like protein 2	SEZ6L2	2.6	2.2	
Delta-like protein 1	DLL1		2.4	
Signal-regulatory protein beta-2	SIRPB2	2.3	2.6	2.0
Macrosialin	CD68	2.6	1.7	

Table 1a

Protein Names	Gene name	Fold upregulation		
		Expt 3	Expt 2	Expt 1
Podocalyxin-like protein 1	PODXL	2.0	2.1	1.8
Platelet glycoprotein 4	CD36	2.3	2.5	
CD70 antigen	CD70	2.6	2.5	3.1
Tumor necrosis factor receptor superfamily member 1A	TNFRSF1A	2.8	2.6	3.1
Neuronal growth regulator 1	NEGR1	3.4		

Table 1b

Protein	Gene ID	Reference NCBI sequence
MRP-1 (ABCC1)	4363	NP_004987.2 GI:134142337
XK	7504	NP_066569.1 GI:10835267
DLL1	28514	NP_005609.3 GI:110735443
CD36	948	NP_000063.2 GI:48375178
TNFRSF1A	7132	NP_001056.1 GI:4507575
NEGR1	257194	NP_776169.2 GI:66346706
UL138	3077420	YP_081578.1 GI:52139303

Table 2

Claims:

1. A method of screening a population of mammalian cells for HCMV infection comprising:
 - providing a population of mammalian cells and
 - determining in one or more cells in the population the expression of multidrug resistance-associated protein 1 (MRP-1)
 - wherein reduced expression of MRP-1 relative to controls is indicative that a cell is infected with HCMV.
2. A method according to claim 1 wherein expression of MRP-1 is determined by;
 - (i) contacting the population of mammalian cells with a binding member which specifically binds to MRP-1 on a cell surface and
 - (ii) determining the binding of the binding member to the cells in the population,
 - wherein a decrease in binding to a cell relative to controls is indicative that the cell has reduced MRP-1 expression.
3. A method according to claim 2 comprising identifying one or more cells in the population to which the binding member shows reduced binding and/or identifying one or more cells in the population to which the binding member does not show reduced binding.
4. A method according to claim 1 wherein expression of MRP-1 is determined by;
 - (i) incubating the population of mammalian cells with a reporter molecule which is selectively expelled from cells by MRP-1,
 - (ii) determining the accumulation of reporter molecule in the cells in the population,
 - wherein an accumulation of the reporter molecule in a cell relative to controls is indicative that the cell has reduced MRP-1 expression.

5. A method according to claim 4 comprising identifying one or more cells in the population in which the reporter molecule accumulates and/or identifying one or more cells in the population in which the reporter molecule does not accumulate.

6. A method according to claim 4 or claim 5 wherein the reporter molecule is seminaphtharhodafluor (SNARF-1).

7. A method according to any one of the preceding claims comprising identifying in the population one or more cells with reduced MRP-1 expression relative to controls, said one or more mammalian cells being HCMV infected.

8. A method according to any one of the preceding claims comprising identifying in the population one or more cells in which MRP-1 expression is not reduced relative to controls, said one or more mammalian cells being non-HCMV infected.

9. A method according to claim 7, or claim 8 wherein the cells identified as HCMV infected cells or non-HCMV infected cells are separated from the population.

10. A method according to claim 9 wherein the separated HCMV infected cells or separated non-HCMV infected cells are stored, retained, cultured and/or expanded.

11. A method of removing HCMV infected cells or non- HCMV infected cells from a population of mammalian cells comprising;

(i) providing a population of mammalian cells

(ii) identifying one or more HCMV infected cells or non-HCMV infected cells in the population using a method according to claim 7 or claim 8,

(iii) removing the identified cells from the population and, optionally,

(iv) repeating steps (ii) and (iii) one or more times.

12. A method according to claim 11 comprising:

(a) identifying one or more cells in the population with reduced expression of MRP-1 relative to controls,

(b) removing the identified cells from the population, and;
optionally repeating steps (a) and (b) one or more times.

13. A method according to claim 11 or claim 12 comprising;

(a) identifying one or more cells in the population which do not have reduced expression of MRP-1 relative to controls,

(b) removing the identified cells from the population, and;
optionally repeating steps (a) and (b) one or more times.

14. A method of depleting HCMV infected cells from a population of mammalian cells comprising:

providing a population of mammalian cells, and;
selectively inducing the death of cells within the population which express reduced amounts of MRP-1 relative to controls,
wherein said one or more cells are HCMV infected cells.

15. A method according to claim 14 wherein cell death is induced by a method comprising;

incubating the population of cells with non-toxic levels of a cytotoxic drug which is selectively expelled from cells by MRP-1,
such that the cytotoxic drug accumulates to toxic levels in said cells which express reduced amounts of MRP-1.

16. A method according to claim 15 wherein the cytotoxic drug is vincristine.

17. A method according to any one of the preceding claims wherein reduced MRP-1 expression relative to controls is indicative that a cell is latently infected with HCMV.

18. A method according to any one of the preceding claims wherein the mammalian cell population is a tissue sample.

19. A method according to claim 18 wherein the tissue is bone marrow.

20. A kit for enriching or depleting a cell population for cells infected with HCMV or cells not infected with HCMV, the kit comprising one or more solid substrates conjugated with antibodies which specifically bind to MRP-1.

21. A kit according to claim 20 wherein the solid substrates are magnetic beads.

22. A method of enriching or depleting a cell population for cells infected with HCMV or cells not infected with HCMV comprising

- (i) contacting the population with one or more immobilised a binding members which bind to MRP-1,
- (ii) separating bound cells from the population and;
- (iii) optionally repeating steps (i) and (ii) one or more times.

23. A method according to claim 22 wherein said binding members bind to MRP-1 and the population of cells which bind to said binding members is depleted of cells infected with HCMV.

24. A population of mammalian cells which has been depleted of cells which are infected with HCMV by a method of any one of the preceding claims.

25. A population according to claim 24 for use as a medicament.

26. A population according to claim 24 for use in the replacement of dysfunctional or damaged tissue in an individual.

27. A method of treating a patient with a damaged or dysfunctional tissue may comprise;

administering population of mammalian cells according to claim 24 to an individual in need thereof.

28. A method of screening for an agent which is useful in depleting or eliminating HCMV infected cells in a population may comprise;

determining the accumulation of a test compound in an MRP-1 deficient cell relative to a non-MRP-1 deficient cell,

wherein increased accumulation in the MRP-1 deficient cells relative to the non-MRP-1 deficient cell is indicative that the compound is useful in identifying, depleting or eliminating HCMV infected cells in a population.

29. A method according to claim 28 wherein the test compound is a cytotoxic agent and the accumulation of the compound in the MRP-1 deficient cells relative to control cells is determined by measuring the amount of cell death in the MRP-1 deficient cells relative to control cells.

30. A method according to claim 28 wherein the test compound is a reporter molecule and the accumulation of the compound in the MRP-1 deficient cells relative to control cells is determined by measuring the amount of reporter molecule in the MRP-1 deficient cells relative to control cells.

31. Use of a binding member which specifically binds to MRP-I in a method of identifying, depleting or eliminating HCMV infected cells in a population of mammalian cells.

32. Use according to claim 31 wherein the method is a method according to any one of claims 1 to 19, 22 or 23.

33. Use according to claim 31 or 32 wherein the binding member is an antibody.

34. Use of a cytotoxic drug which is selectively expelled from cells by MRP-1 in a method of identifying, depleting or eliminating HCMV infected cells in a population of mammalian cells.

35. Use according to claim 34 wherein the method is a method according to any one of claims 1 to 19, 22, 23 or 28 to 30.

36. Use according to claim 34 or 35 wherein the cytotoxic drug is vincristine.

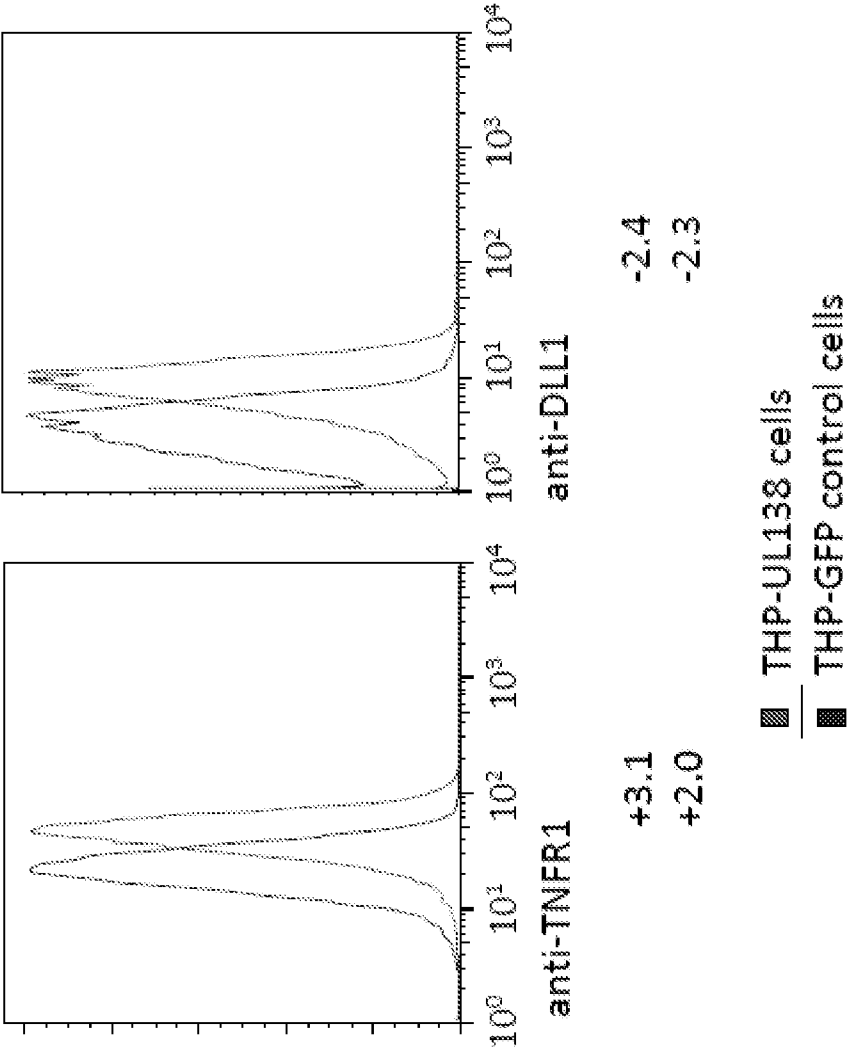


Figure 1

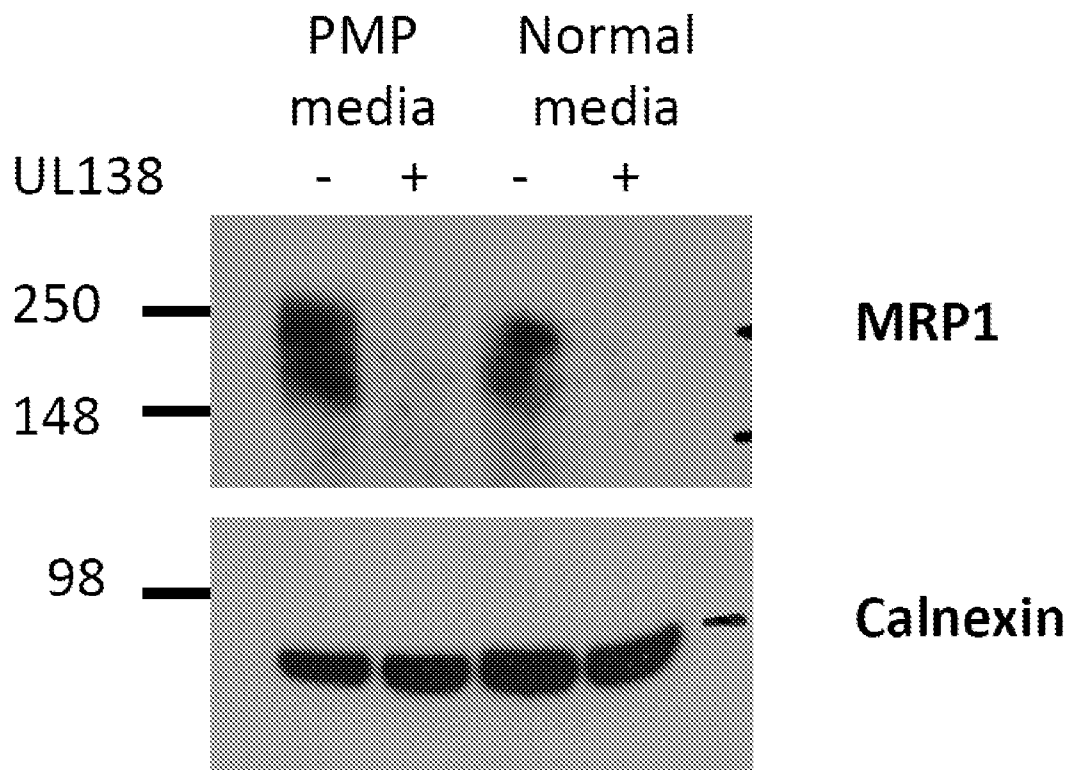


Figure 2

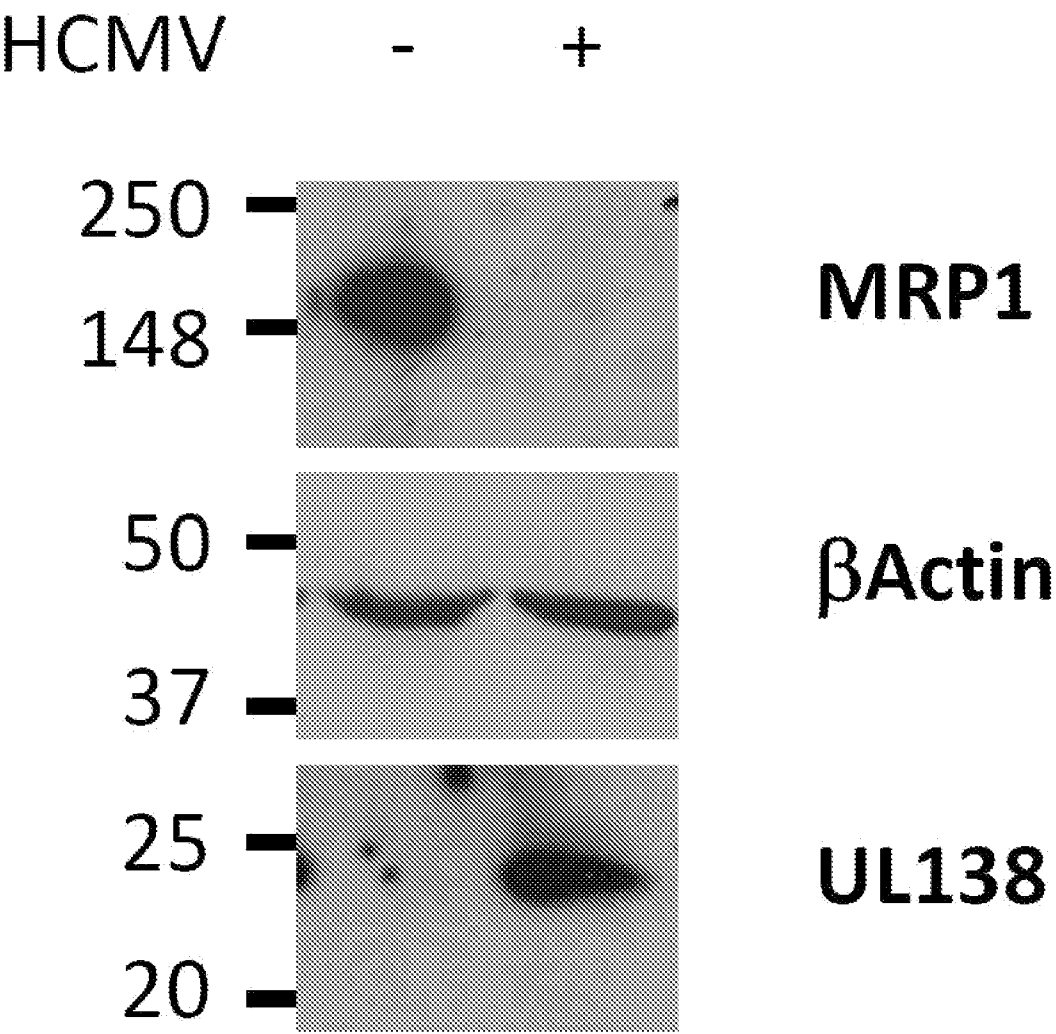


Figure 3

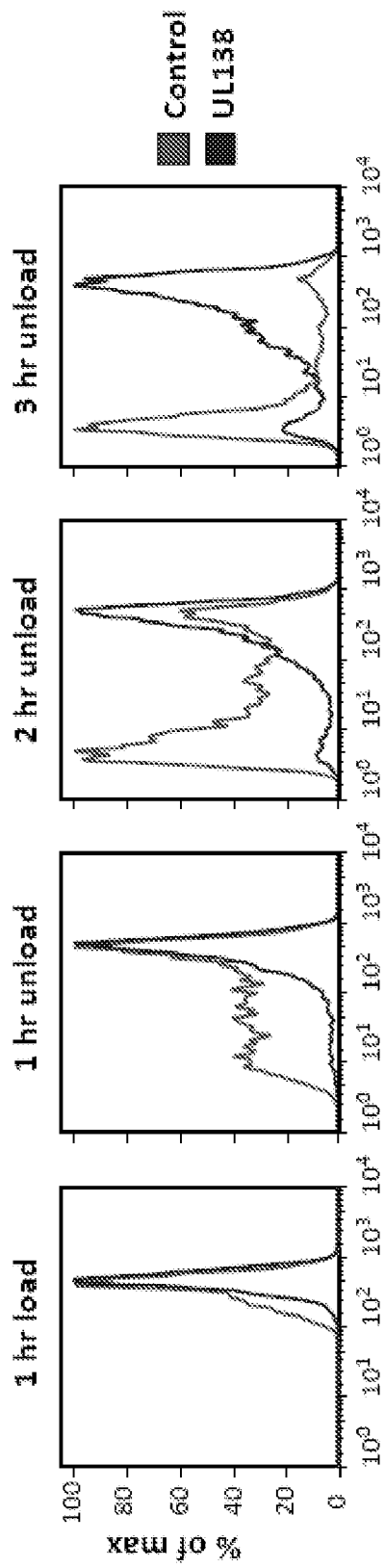


Figure 4

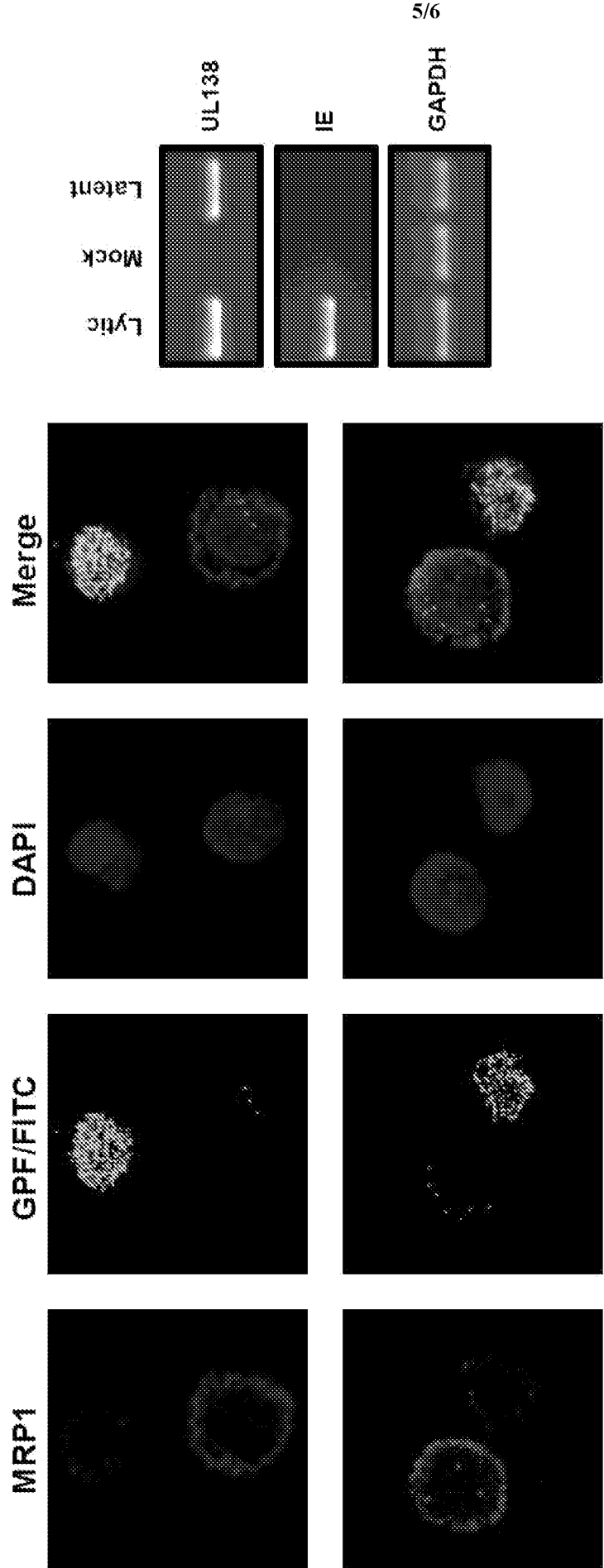


Figure 5

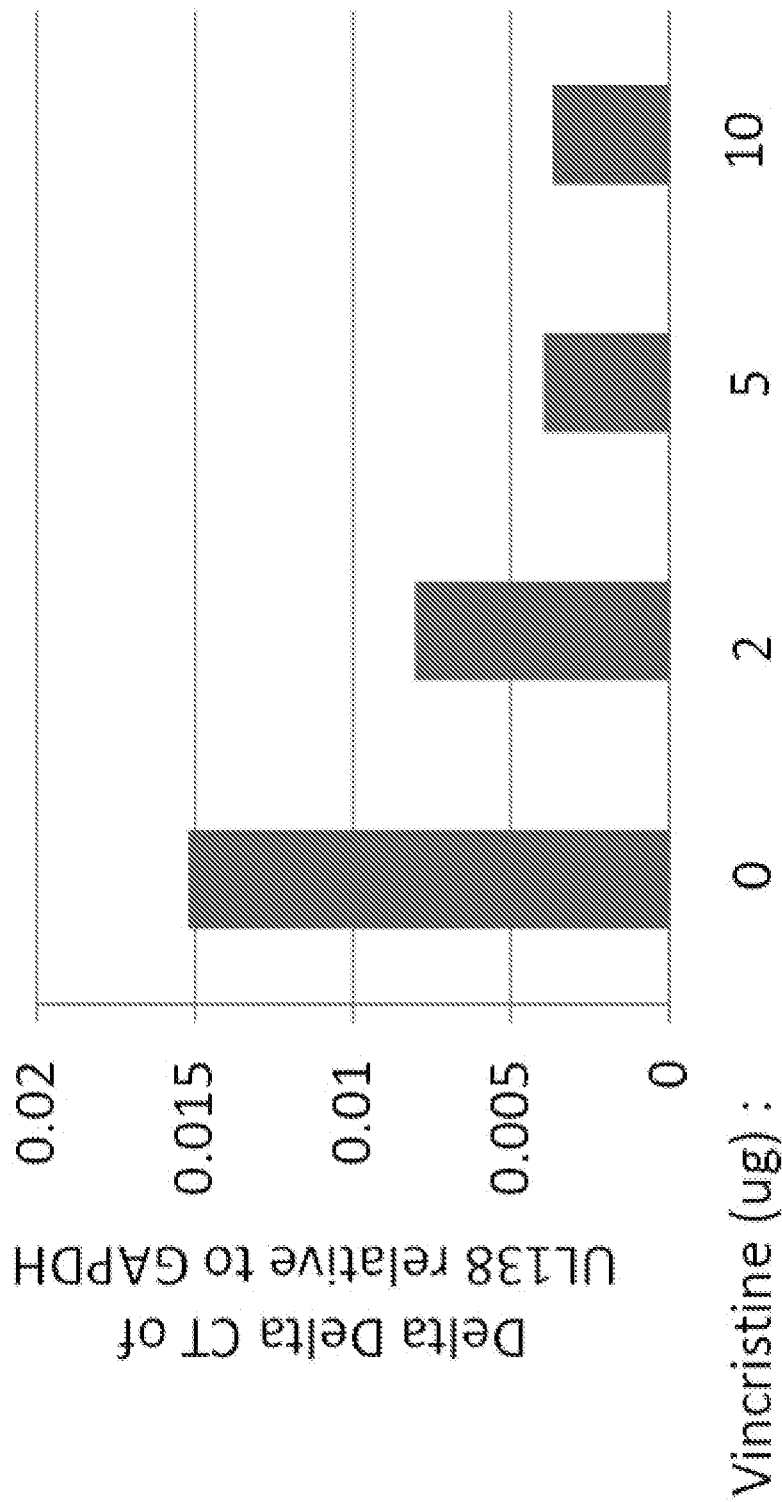


Figure 6

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2012/051094

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/569 G01N33/50 G01N33/68 A61L2/00 A61K45/06
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N A61K

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/059142 A2 (EPIDAUROS BIOTECHNOLOGIES AG [DE]; BRINKMANN ULRICH [DE]; HOFFMEYER SV) 1 August 2002 (2002-08-01) abstract; claims	20,21
X	WO 02/062956 A2 (CHEMOCENTRYX INC [US]; SCHALL THOMAS J [US]; PENFOLD MARK E T [US]) 15 August 2002 (2002-08-15) the whole document	24-27

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

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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

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

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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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

"&" document member of the same patent family

Date of the actual completion of the international search

30 August 2012

Date of mailing of the international search report

07/09/2012

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Stricker, J

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2012/051094

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	----- WO 01/37868 A1 (OKLAHOMA MED RES FOUND [US]) 31 May 2001 (2001-05-31)	36
A	abstract; claims page 14, line 15 - page 16, line 2 page 19, line 17 - page 24, line 18	1-35
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