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(54) Title: HYBRIDOMAS PRODUCING ANTIBODIES AGAINST NON FUNCTIONAL P2X7 RECEPTOR

(57) Abstract: The present invention relates to the production of anti non functional P2X7 receptor monoclonal antibodies from hybridoma cell lines.



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1.

Hybridomas producing antibodies against non functional P2X7 receptor**FIELD OF THE INVENTION**

The present invention relates to the production of monoclonal antibodies from hybridoma cell lines.

5 BACKGROUND OF THE INVENTION

Purinergic (P2X) receptors are ATP-gated cation –selective channels. Each receptor is made up of three protein subunits or monomers. To date seven separate genes encoding P2X monomers have been identified: P2X1, P2X2, P2X3, P2X4, P2X5, P2X6, P2X7.

10 P2X7 receptors are of particular interest as the expression of these receptors is understood to be limited to cells having potential to undergo programmed cell death, such as thymocytes, dendritic cells, lymphocytes, macrophages and monocytes. There is some expression of P2X7 receptors in normal homeostasis, such as on erythrocytes.

15 Interestingly, a P2X7 receptor containing one or more monomers having a cis isomerisation at Pro210 (SEQ ID NO: 1) and which is devoid of ATP binding function has been found on cells that are understood to be unable to undergo programmed cell death, such as preneoplastic cells and neoplastic cells. This isoform of the receptor has been referred to as a “non functional” receptor.

20 Antibodies generated from immunisation with a peptide including Pro210 in cis bind to non functional P2X7 receptors. However, they do not bind to P2X7 receptors capable of binding ATP. Accordingly, these antibodies are useful for selectively detecting many forms of carcinoma and haemopoietic cancers and to treatment of some of these conditions.

WO02/057306A1 and WO03/020762A1 both discuss a probe for distinguishing between functional P2X7 receptors and non functional P2X7 receptors in the form of a monoclonal antibody.

2.

To date it has been very difficult to obtain a hybridoma that generates useful amounts of antiserum against non functional P2X7 receptors, and in particular, antiserum that can be used in a range of diagnostic and therapeutic applications. Indeed, apart from the hybridomas and antibodies forming part of this invention, the Applicant is unaware of any other hybridomas or
5 monoclonal antibodies against anti- non functional P2X7 receptors that could be robustly used in applications to detect or treat cancer and other conditions associated with non functional P2X7 receptor expression.

SUMMARY OF THE INVENTION

In one embodiment there is provided a hybridoma for producing an antibody that is capable of
10 forming an immune complex with a non functional P2X7 receptor, the hybridoma being characterised in that it produces an antibody that has an affinity for non functional P2X7 receptors expressed on a live cell of less than about $5 \times 10^6 \text{ M}^{-1}$.

In another embodiment there is provided an antibody produced by a hybridoma described above.

In other embodiments there is provided a fragment of a monoclonal antibody described above
15 that is capable of forming an immune complex with a non functional P2X7 receptor.

In other embodiments there is provided an immune complex formed from the binding of an antibody or fragment described above to a non functional P2X7 receptor, monomer or fragment thereof, or to a peptide having a sequence shown in SEQ ID NO:2.

In certain embodiments there is provided a method for determining whether a cell, tissue or extra
20 cellular body fluid includes a non functional P2X7 receptor, monomer or fragment thereof including:

- contacting a cell, tissue or extra cellular body fluid with an antibody or fragment described above in conditions for forming an immune complex as described above, and
- detecting whether an immune complex has been formed,

3.

wherein detection of an immune complex determines that a cell, tissue or extra -cellular body fluid includes a non functional P2X7 receptor, monomer or fragment thereof.

In yet further embodiments there is provided a kit or composition for determining whether a cell, tissue or extra -cellular body fluid contains a non functional P2X7 receptor, monomer or
5 fragment thereof including:

- an antibody or fragment described above and optionally;
- a further antibody for binding to the antibody or fragment or the non functional P2X7 receptor, monomer or fragment thereof;
- written instructions for use of the kit in a method described above.

- 10 In other embodiments there is provided a pharmaceutical composition including an antibody or fragment as described above together with a pharmaceutically acceptable carrier, diluent or excipient.

- In related embodiments there is provided a method of treatment of a disease characterised by the expression of a non ATP -binding P2X7 receptor, monomer or fragment thereof including the
15 step of providing an antibody or fragment thereof as described above, or a peptide as described above to an individual requiring said treatment.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows BPM09 immunohistochemical staining of human prostate cancer tissue.

- Figure 2** shows (A) BPM09 competition of biotinylated BPM09, and (B) isotype control
20 competition of biotinylated BPM09.

DETAILED DESCRIPTION OF THE INVENTION

4.

The anti P2X7 antisera against non functional P2X7 receptors available at the time of the invention have all been polyclonal. Apart from the Applicant's own work, no anti- non functional P2X7 receptor monoclonal antibodies have been made.

5 The inventors have attempted to obtain anti non functional P2X7 receptor monoclonal antibodies using techniques for monoclonal antibody production known in the art. In accordance with conventional techniques, a key step in this process has been to screen and to select for hybridomas for antibody production that produce supernatants having high affinity for the peptide immunogen against which they have been raised (Goding James W. Monoclonal antibodies: principles and practice: production and application of monoclonal antibodies in cell
10 biology, biochemistry and immunology. – 2nd ed. 1986 Academic Press, Harcour Brace Jovnovich, Publishers).

In forming this invention, the inventors found that these techniques tend to result in hybridomas that exhibit poor growth in mice and that are sensitive to cell culture techniques including passaging, freezing, thawing and seeding. Consequently the inventors have found it to be very
15 difficult to generate useful amounts of antibody from hybridomas selected according to the conventional monoclonal antibody production techniques.

Further, the inventors have found that the hybridomas selected for antibody production on the basis of high affinity binding to peptide immunogen tend to produce antibodies that also have high affinity for non functional P2X7 receptors expressed on the surface of live cells. The
20 inventors now believe that these antibodies bind to the hybridomas that produce them with high affinity.

Still further, in forming this invention the inventors have screened for hybridomas that secrete antibodies that bind to non functional P2X7 receptors expressed on live cells with low affinity, and then determined the capacity of these hybridoma cells to grow in mice and tissue culture.
25 The inventors have surprisingly found that the hybridomas showed a much better potential for growth and stability than those that secrete antibodies that bind to receptors on live cells with high affinity. As a result, the inventors have been able to produce large amounts of monoclonal antibody to non functional P2X7 receptors.

5.

Further, the inventors have surprisingly found that the antibodies having a low affinity for non functional receptors expressed on live cells have a high affinity for non functional receptors expressed on dead or fixed cells or tissues, such as fixed cells that are routinely screened in FACS and paraffin tissue sections. Accordingly, the inventors have been able to produce large amounts of monoclonal antibody useful in a wide range of diagnostic applications not involving live cells, such as FACS, histology and various other cell -free serological applications including ELISA, RIA and the like.

Thus, in one embodiment there is provided a hybridoma for producing an antibody that is capable of forming an immune complex with a non functional P2X7 receptor, the hybridoma being characterised in that it produces an antibody that has an affinity for non functional P2X7 receptors expressed on a live cell of less than about $5 \times 10^6 \text{ M}^{-1}$, preferably less than about $5 \times 10^5 \text{ M}^{-1}$, more preferably about $5 \times 10^4 \text{ M}^{-1}$ or less, or in the range of from $5 \times 10^4 \text{ M}^{-1}$ to $5 \times 10^5 \text{ M}^{-1}$. In one embodiment, the hybridoma is characterised in that it produces an antibody that has an affinity that is about the same as the affinity of antibody BPM09 disclosed herein for a non functional receptor expressed on a live cell. An example of a live cell is a myeloma or hybridoma cell. Other live cells or cell lines include those expressing non functional P2X7 receptor, such as prostate PC3.

One example of a hybridoma according to this embodiment of the invention is hybridoma AB253 deposited with the European Collection of Cell Cultures (ECACC) under Accession no. 06080101. The inventors have found that this hybridoma is stable in the sense that it grows and consistently produces antiserum against non function P2X7 receptors after passaging and freeze/thawing. Thus in one embodiment there is provided hybridoma AB253 deposited with the European Collection of Cell Cultures (ECACC) under Accession no. 06080101. A sample of the hybridoma AB253 was deposited with (ECACC), Porton Down, Salisbury, Wiltshire, SP4 0JG, United Kingdom, on 1 August 2006.

In another embodiment there is provided an antibody produced by a hybridoma described above. Monoclonal antibody BPM09, which is produced by the hybridoma AB253 deposited with the European Collection of Cell Cultures (ECACC) under Accession no. 06080101 is an example of a monoclonal antibody according to this embodiment. This antibody is distinguished from other tumour-selective P2X₇ polysera, in particular in respect to its superior immunohistochemical

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staining of tumour tissue. Labelled BPM09 antibodies bind strongly to tumour tissue, with minimal staining of normal tissue, when compared to other anti-P2X₇ tumour selective polyclonal antibodies.

In further embodiments, a monoclonal antibody produced by a hybridoma of the invention is one which is raised against a peptide having a sequence shown in SEQ ID NO: 2. Examples include the following monoclonal antibodies: BPM01, BPM02, BPM03, BPM04, BPM05, BPM06, BPM07, BPM08, BPM10, also further described herein. In other embodiments, a monoclonal antibody produced by a hybridoma of the invention is one which is raised against a peptide derived from elsewhere in the extracellular domain or other domain of a non functional P2X₇ receptor.

As known in the art, an "antibody" is an immunoglobulin molecule. Five isotypes are known: IgG, IgA, IgM, IgD and IgE. All antibodies (except for IgM) are comprised of four polypeptide chains in the form of two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain is comprised of a light chain variable region (LCVR or V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. IgM has the above structure, however it is pentameric. In one embodiment, the monoclonal antibody is an IgG1 isotype.

The term "domain" as used herein is meant a folded protein structure which retains its tertiary structure independently of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins, and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain.

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The term "variable domain" as used herein is meant a folded polypeptide domain comprising sequences characteristic of immunoglobulin heavy or light chain variable domains and which specifically binds an antigen.

5 The term "immunoglobulin" as used herein refers to a family of polypeptides which retain the immunoglobulin fold characteristic of antibody molecules, which contains two β sheets and, usually, a conserved disulphide bond. Members of the immunoglobulin superfamily are involved in many aspects of cellular and non-cellular interactions in vivo, including widespread roles in the immune system (for example, antibodies, T-cell receptor molecules and the like), involvement in cell adhesion (for example the ICAM molecules) and intracellular signalling (for
10 example, receptor molecules, such as the PDGF receptor).

In other embodiments there is provided a fragment of a monoclonal antibody described above that is capable of forming an immune complex with non functional P2X7 receptor. These fragments are typically formed by a chemical reaction or modification of a monoclonal antibody. One example is a Fab fragment which is formed by papain digestion of whole antibody. The
15 fragments typically retain the same affinity for antigen as the whole antibody from which they are derived.

The antibody or fragment may be provided on a solid phase such as a bead, surface or tissue culture vessel.

20 The antibody or fragment may be provided with a label for detection of binding of the antibody or fragment to antigen.

The antibodies and fragments may be labelled for use in medical imaging. Such methods involve chemical attachment of a labelling or imaging agent, such as a radioisotope, which include ^{67}Cu , ^{90}Y , ^{125}I , ^{131}I , ^{186}Re , ^{188}Re , ^{211}At , ^{212}Bi , administration of the labelled antibody or fragment to a subject in an acceptable carrier, and imaging the labelled antibody or fragment *in vivo* at the target site. Radio-labelled antibodies or fragments thereof may be particularly useful
25 in *in vivo* imaging of cancers described herein.

8.

The antibodies can be purified by methods known to the skilled artisan. Purification methods include, among other, selective precipitation, liquid chromatography, HPLC, electrophoresis, chromatofocusing, and various affinity techniques.

5 In some embodiments, the antibodies disclosed herein may also include multimeric forms of antibodies. For example, antibodies of the invention may take the form of antibody dimers, trimers, or higher-order multimers of monomeric immunoglobulin molecules.

Crosslinking of antibodies can be done through various methods known in the art. For example, crosslinking of antibodies may be accomplished through natural aggregation of antibodies, through chemical or recombinant linking techniques or other methods known in the art. For
10 example, purified antibody preparations can spontaneously form protein aggregates containing antibody homodimers, and other higher-order antibody multimers. In a specific embodiment, crosslinking of antibodies by using a second antibody to bind to the antibodies of interest can be used to form a homodimer. The crosslinker antibody can be derived from a different animal compared to the antibody of interest. For example, a goat anti- mouse antibody (Fab specific)
15 may be added to a mouse monoclonal antibody to form a homodimer. This bivalent crosslinker antibody recognizes the Fab or Fc region of the two antibodies of interest forming a homodimer.

Alternatively, antibody homodimers may be formed through chemical linkage techniques known in the art. Chemical crosslinkers can be homo or heterobifunctional and will covalently bind with two antibodies forming a homodimer. In some embodiments, it is desirable that the chemical
20 crosslinker not interact with the antigen-binding region of the antibody as this may affect antibody function. As will be appreciated by those skilled in the art, antibodies can be crosstinked at the Fab region.

In other embodiments there is provided an immune complex formed from the binding of an antibody or fragment described above to a non functional P2X7 receptor, monomer or fragment
25 thereof, or to a peptide shown in SEQ ID NO 2.

The immune complex, otherwise known as an antibody-antigen complex, or a complex formed from the binding of an antigen binding site of an antibody or fragment thereof to an antigenic determinant or epitope, is particularly important as detection of this *in vitro* or *in vivo* is

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indicative of presence of, or predisposition to a disease or condition including preneoplasia and neoplasia. These detection methods are described in more detail below.

The P2X7 receptor, monomer or fragment thereof included in the immune complex may have Pro210 in *cis* isomerisation.

- 5 The P2X7 receptor, monomer or fragment thereof included in the immune complex may have an amino acid sequence as shown in any one of SEQ ID NO:s. 3 to 8 or fragment thereof.

The P2X7 receptor, monomer or fragment thereof included in the immune complex may have a molecular weight in the range of from about 15 to 80 kDa, not including the molecular weight of the antibody or antibody fragment. The total molecular weight depends on whether the complex
10 is formed from a whole antibody or fragment thereof.

The P2X7 receptor, monomer or fragment thereof included in the immune complex may lack a transmembrane domain..

The immune complex may be formed by binding a P2X7 receptor, monomer or fragment thereof located on a cell surface membrane, in a cytoplasm, in a nucleus or in extra-cellular fluid. The
15 extra -cellular fluid may be blood, plasma, serum, lymph, urine, semen, saliva, sputum, ascites, faeces, uterine and vaginal secretions, bile, amniotic fluid, cerebrospinal fluid and organ and tissue flushings.

The antibody or antibody fragment included in the immune complex may be attached to a solid phase, such as a bead or a plate, so that the immune complex is attached to a solid phase when
20 formed. Alternatively, the P2X7 receptor, monomer or fragment thereof included in the immune complex may be attached to a solid phase.

The antibody may be labelled for detection of formation of the immune complex.

The immune complex may further include an antibody or fragment thereof, such as a capture antibody for capture of the immune complex. The further antibody or fragment thereof may bind
25 to the anti P2X7 receptor antibody. Also, the further antibody or fragment thereof may bind to the receptor or fragment thereof.

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The further antibody or fragment thereof may be bound to a solid phase such as a phase described above.

The further antibody may be labelled for detection of formation of the immune complex. Examples of labels include fluorophores, dyes, isotopes etc.

- 5 In certain embodiments there is provided a method for determining whether a cell, tissue or extra cellular body fluid includes a non functional P2X7 receptor, monomer or fragment thereof including:

- contacting a cell, tissue or extra cellular body fluid with an antibody or fragment described above in conditions for forming an immune complex as described above, and

- 10 - detecting whether an immune complex has been formed,

wherein detection of an immune complex determines that a cell, tissue or extra -cellular body fluid includes a non functional P2X7 receptor, monomer or fragment thereof.

In other embodiments there is provided a use of an antibody or fragment thereof described above in the manufacture of means for determining whether a cell, tissue or extra-cellular body fluid

- 15 contains a P2X7 receptor, monomer or fragment thereof.

The presence of a given protein, or level of expression of a given protein in a host cell, tissue or extra -cellular body fluid can be detected by any number of assays. Examples include immunoassays, chromatography and mass spectrometry.

Immunoassays, i.e. assays involving an element of the immune system are particularly preferred.

- 20 These assays may generally be classified into one of:

(i) assays in which purified antigen is used to detect an antibody in host serum. For example, purified antigen is bound to solid phase by adsorption or indirectly through another molecule and host serum is applied followed by another antibody for detecting presence or absence of host antibody;

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(ii) assays in which purified antigen is used to detect immune cells, such as T and B lymphocytes. For example, peripheral white cells are purified from a host and cultured with purified antigen. The presence or absence of one or factors indicating immunity are then detected. Other examples include assays that measure cell proliferation (lymphocyte proliferation or transformation assays) following exposure to purified antigen, and assays that measure cell death (including apoptosis) following exposure to purified antigen;

(iii) assays in which purified antibody specific for antigen is used to detect antigen in the host. For example, purified antibody is bound to solid phase, host tissue is then applied followed by another antibody specific for the antigen to be detected. There are many examples of this approach including ELISA, RIA;

(iv) assays in which a purified anti-idiotypic antibody is used to detect host antibody. For example, anti-idiotypic antibody is adsorbed to solid phase, host serum is added and anti-Fc antibody is added to bind to any host antibodies having been bound by the anti-idiotypic antibody.

15 The immunoassays can be applied *in vitro* or *in vivo*.

In one embodiment, the disease is typically a cancer such as carcinoma, sarcoma, lymphoma, or leukemia. Carcinomas that may be detected include, but not limited to, prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, and colon cancers.

20 Whilst any body fluid can be used to detect any of these diseases, some body fluids may be more appropriate than others to detect certain diseases, for example urine may be more appropriate to detect prostate cancer and blood for detecting blood cancers such as lymphoma.

In certain embodiments, cancer is selected from the group consisting of prostate cancer, invasive breast cancer, melanoma, adenocarcinoma of the bowel, serous ovarian cancer, squamous cell cancer of the cervix, endometrial cancer, small cell lung cancer, hepatocellular carcinoma, transitional cell carcinoma of the bladder, gastrointestinal stromal tumour, endometrial stromal tumour, pituitary cancer, mesothelioma, Hodgkin's lymphoma and thyroid papillary.

12.

In yet further embodiments there is provided a kit or composition for determining whether a cell, tissue or extra -cellular body fluid contains a non functional P2X7 receptor, monomer or fragment thereof including:

- an antibody or fragment described above and optionally;
- 5 - a further antibody for binding to the antibody or fragment or the non functional P2X7 receptor, monomer or fragment thereof;
- written instructions for use of the kit in a method described above.

Kits are provided which contain the necessary reagents to carry out the assays of the present invention. The kit may include one or more compartments, each to receive one or more
10 containers such as: (a) a first container comprising one of the components of the present invention described above; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of the antibody or peptide.

The containers allow one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or
15 solutions of each container can be added in a quantitative fashion from one compartment to another.

The kit typically contains containers which may be formed from a variety of materials such as glass or plastic, and can include for example, bottles, vials, syringes, and test tubes. A label typically accompanies the kit, and includes any writing or recorded material, which may be
20 electronic or computer readable form (e.g., disk, optical disc, or tape) providing instructions or other information for use of the contents of the kit. The label indicates that the formulation is used for diagnosing or treating the disorder of choice.

One skilled in the art will readily recognize that the disclosed antibodies and peptides of the present invention can be readily incorporated into one of the established kit formats which are
25 well known in the art.

13.

In other embodiments there is provided a pharmaceutical composition including an antibody or fragment as described above together with a pharmaceutically acceptable carrier, diluent or excipient.

In the preparation of the pharmaceutical compositions comprising the antibodies or peptides described in the teachings herein, a variety of vehicles and excipients and routes of administration may be used, as will be apparent to the skilled artisan. Representative formulation technology is taught in, inter alia, Remington: The Science and Practice of Pharmacy, 19th Ed., Mack Publishing Co., Easton, PA (1995) and Handbook of Pharmaceutical Excipients, 3rd Ed, Kibbe, A.H. ed., Washington DC, American Pharmaceutical Association (2000); hereby
5
10 incorporated by reference in their entirety.

The pharmaceutical compositions will generally comprise a pharmaceutically acceptable carrier and a pharmacologically effective amount of the antibodies or peptides, or mixture of antibodies or mixture of peptides, or suitable salts thereof.

The pharmaceutical composition may be formulated as powders, granules, solutions, suspensions, aerosols, solids, pills, tablets, capsules, gels, topical creams, suppositories, transdermal patches, and other formulations known in the art.
15

For the purposes described herein, pharmaceutically acceptable salts of the antibodies and peptides is intended to include any art recognized pharmaceutically acceptable salts including organic and inorganic acids and/or bases. Examples of salts include sodium, potassium, lithium, ammonium, calcium, as well as primary, secondary, and tertiary amines, esters of lower hydrocarbons, such as methyl, ethyl, and propyl. Other salts include organic acids, such as acetic acid, propionic acid, pyruvic acid, maleic acid, succinic acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, salicylic acid, etc.
20

As used herein, "pharmaceutically acceptable carrier" comprises any standard pharmaceutically accepted carriers known to those of ordinary skill in the art in formulating pharmaceutical compositions. Thus, the antibodies or peptides, by themselves, such as being present as pharmaceutically acceptable salts, or as conjugates, may be prepared as formulations in pharmaceutically acceptable diluents; for example, saline, phosphate buffer saline (PBS),
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aqueous ethanol, or solutions of glucose, mannitol, dextran, propylene glycol, oils (e.g., vegetable oils, animal oils, synthetic oils, etc.), microcrystalline cellulose, carboxymethyl cellulose, hydroxylpropyl methyl cellulose, magnesium stearate, calcium phosphate, gelatin, polysorbate 80 or as solid formulations in appropriate excipients.

- 5 The pharmaceutical compositions will often further comprise one or more buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxytoluene, butylated hydroxyanisole, etc.), bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminium hydroxide), solutes
10 that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate.

- While any suitable carrier known to those of ordinary skill in the art may be employed in the compositions of this invention, the type of carrier will typically vary depending on the mode of
15 administration. Antibody and peptide compositions may be formulated for any appropriate manner of administration, including for example, oral, nasal, mucosal, intravenous, intraperitoneal, intradermal, subcutaneous, and intramuscular administration.

- For parenteral administration, the compositions can be administered as injectable dosages of a solution or suspension of the substance in a physiologically acceptable diluent with a
20 pharmaceutical carrier that can be a sterile liquid such as sterile pyrogen free water, oils, saline, glycerol, polyethylene glycol or ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, surfactants, pH buffering substances and the like can be present in compositions.

- Other components of pharmaceutical compositions are those of petroleum, animal, vegetable, or
25 synthetic origin, for example, non-aqueous solutions of peanut oil, soybean oil, corn oil, cottonseed oil, ethyl oleate, and isopropyl myristate. Antibodies and peptides can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained release of the active ingredient. An exemplary

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composition comprises antibody at 5 mg/ml, formulated in aqueous buffer consisting of 50 mM L-histidine, 150 mM NaCl, adjusted to pH 6.0 with HCl.

Typically, the compositions are prepared as injectables, either as liquid solutions or suspensions; solid or powder forms suitable for reconstitution with suitable vehicles, including by way
5 example and not limitation, sterile pyrogen free water, saline, buffered solutions, dextrose solution, etc., prior to injection. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymers.

The pharmaceutical compositions described herein may be presented in unit-dose or multi- dose containers, such as sealed ampoules or vials. Such containers are typically sealed in such a way
10 to preserve the sterility and stability of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles, as indicated above.

Alternatively, a pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

In related embodiments there is provided a method of treatment of a disease characterised by the
15 expression of a non ATP –binding P2X7 receptor, monomer or fragment thereof including the step of providing an antibody or fragment thereof as described above, or a peptide as described above to an individual requiring said treatment.

Methods of immunotargeting cancer cells using antibodies or antibody fragments are well known in the art. U.S. Pat. No. 6,306,393 describes the use of anti-CD22 antibodies in the
20 immunotherapy of B-cell malignancies, and U.S. Pat. No. 6,329,503 describes immunotargeting of cells that express serpentine transmembrane antigens. Antibodies described herein (including humanized or human monoclonal antibodies or fragments or other modifications thereof, optionally conjugated to cytotoxic agents) can be introduced into a patient such that the antibody binds to cancer cells and mediates the destruction of the cells and the tumor and/or inhibits the
25 growth of the cells or the tumor.

Without intending to limit the disclosure, mechanisms by which such antibodies can exert a therapeutic effect may include complement-mediated cytotoxicity, antibody-dependent cellular cytotoxicity (ADCC)1 modulating the physiologic function of the tumor antigen, inhibiting

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binding or signal transduction pathways, modulating tumor cell differentiation, altering tumor angiogenesis factor profiles, modulating the secretion of immune stimulating or tumor suppressing cytokines and growth factors, modulating cellular adhesion, and/or by inducing apoptosis.

- 5 The antibodies can also be conjugated to toxic or therapeutic agents, such as radioligands or cytosolic toxins, and may also be used therapeutically to deliver the toxic or therapeutic agent directly to tumor cells.

By "treatment" herein is meant therapeutic or prophylactic treatment, or a suppressive measure for the disease, disorder or undesirable condition. Treatment encompasses administration of the
10 subject antibodies in an appropriate form prior to the onset of disease symptoms and/or after clinical manifestations, or other manifestations, of the disease to reduce disease severity, halt disease progression, or eliminate the disease. Prevention of the disease includes prolonging or delaying the onset of symptoms of the disorder or disease, preferably in a subject with increased susceptibility to the disease.

- 15 The therapeutic preparations can use nonmodified antibodies or antibodies conjugated with a therapeutic compound, such as a toxin or cytotoxic molecule, depending on the functionality of the antibody. Generally, when nonmodified antibodies are used, they will typically have a functional Fc region. By "functional Fc region" herein is meant a minimal sequence for effecting the biological function of Fc, such as binding to Fc receptors, particularly FcγR (e.g.,
20 FcγRI, FcγRII, and FcγRIII).

Without being bound by theory, it is believed that the Fc region may affect the effectiveness of anti-tumor monoclonal antibodies by binding to Fc receptors immune effector cells and modulating cell mediated cytotoxicity, endocytosis, phagocytosis, release of inflammatory cytokines, complement mediated cytotoxicity, and antigen presentation. In this regard, polyclonal
25 antibodies, or mixtures of monoclonals will be advantageous because they will bind to different epitopes and thus have a higher density of Fc on the cell surface as compared to when a single monoclonal antibody is used. Of course, to enhance their effectiveness in depleting targeted cells, or where nonmodified antibodies are not therapeutically effective, antibodies conjugated to toxins or cytotoxic agents may be used.

17.

The antibody compositions may be used either alone or in combination with other therapeutic agents to increase efficacy of traditional treatments or to target abnormal cells not targeted by the antibodies. Combining the antibody therapy method with a chemotherapeutic, radiation or surgical regimen may be preferred in patients that have not received chemotherapeutic treatment, 5 whereas treatment with the antibody therapy may be indicated for patients who have received one or more chemotherapies. Additionally, antibody therapy can also enable the use of reduced dosages of concomitant chemotherapy, particularly in patients that do not tolerate the toxicity of the chemotherapeutic agent very well. Furthermore, treatment of cancer patients with the antibody with tumors resistant to chemotherapeutic agents might induce sensitivity and 10 responsiveness to these agents in combination.

In one aspect, the antibodies are used adjunctively with therapeutic cytotoxic agents, including, by way of example and not limitation, busulfan, thioguanine, idarubicin, cytosine arabinoside, 6-mercaptopurine, doxorubicin, daunorubicin, etoposide, and hydroxyurea. Other agents useful as 15 adjuncts to antibody therapy are compounds directed specifically to the abnormal cellular molecule found in the disease state. These agents will be disease specific. For example, for treating chronic myeloid leukemia arising from BCR-ABL activity, one class of useful compounds are inhibitors of abl kinase activity, such as Imatinib, an inhibitor of bcr-abl kinase, and antisense oligonucleotides against bcr (e.g., Oblimersen). Other agents include, among 20 others, interferon-alpha, humanized anti- CD52, deacetylase inhibitor FR901228 (depsipeptide), and the like.

The amount of the compositions needed for achieving a therapeutic effect will be determined empirically in accordance with conventional procedures for the particular purpose. Generally, for administering the compositions ex vivo or in vivo for therapeutic purposes, the compositions are given at a pharmacologically effective dose. By "pharmacologically effective amount" or 25 "pharmacologically effective dose" is an amount sufficient to produce the desired physiological effect or amount capable of achieving the desired result, particularly for treating or retreating the disorder or disease condition, including reducing or eliminating one or more symptoms or manifestations of the disorder or disease.

As an illustration, administration of antibodies to a patient suffering from prostate cancer 30 provides a therapeutic benefit not only when the underlying disease is eradicated or ameliorated,

18.

but also when the patient reports a decrease in the severity or duration of the symptoms associated with the disease. Therapeutic benefit also includes halting or slowing the progression of the underlying disease or disorder, regardless of whether improvement is realized.

The amount administered to the host will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy, the state of the host, the manner of administration, the number of administrations, interval between administrations, and the like. These can be determined empirically by those skilled in the art and may be adjusted for the extent of the therapeutic response. Factors to consider in determining an appropriate dose include, but is not limited to, size and weight of the subject, the age and sex of the subject, the severity of the symptom, the stage of the disease, method of delivery of the agent, half-life of the agents, and efficacy of the agents. Stage of the disease to consider includes whether the disease is acute or chronic, relapsing or remitting phase, and the progressiveness of the disease. Determining the dosages and times of administration for a therapeutically effective amount are well within the skill of the ordinary person in the art.

For any compositions of the present disclosure, the therapeutically effective dose is readily determined by methods well known in the art. For example, an initial effective dose can be estimated from cell culture or other in vitro assays. For example, Sliwkowsky, MX et al., *Semin. Oncol.* 26.suppl. 12) 60-70 (1999) describes in vitro measurements of antibody dependent cellular cytotoxicity. A dose can then be formulated in animal models to generate a circulating concentration or tissue concentration, including that of the IC₅₀ as determined by the cell culture assays.

In addition, the toxicity and therapeutic efficacy are generally determined by cell culture assays and/or experimental animals, typically by determining a LD₅₀ (lethal dose to 50% of the test population) and ED₅₀ (therapeutically effectiveness in 50% of the test population). The dose ratio of toxicity and therapeutic effectiveness is the therapeutic index. Preferred are compositions, individually or in combination, exhibiting high therapeutic indices. Determination of the effective amount is well within the skill of those in the art, particularly given the detailed disclosure provided herein. Guidance is also found in standard reference works, for example Fingl and Woodbury, *General Principles In: The Pharmaceutical Basis of Therapeutics* pp. 1-46 (1975), and the references cited therein.

19.

To achieve an initial tolerizing dose, consideration is given to the possibility that the antibodies may be immunogenic in humans and in non-human primates. The immune response may be biologically significant and may impair the therapeutic efficacy of the antibody even if the antibody is partly or chiefly comprised of human immunoglobulin sequences such as, for example, in the case of a chimeric or humanized antibody. Within certain embodiments, an initial high dose of antibody is administered such that a degree of immunological tolerance to the therapeutic antibody is established.

The tolerizing dose is sufficient to prevent or reduce the induction of an antibody response to repeat administration of the committed progenitor cell specific antibody.

- 10 Preferred ranges for the tolerizing dose are between 10 mg/kg body weight to 50 mg/kg body weight, inclusive. More preferred ranges for the tolerizing dose are between 20 and 40 mg/kg, inclusive. Still more preferred ranges for the tolerizing dose are between 20 and 25 mg/kg, inclusive.

- 15 Within these therapeutic regimens, the therapeutically effective dose of antibodies is preferably administered in the range of 0.1 to 10 mg/kg body weight, inclusive. More preferred second therapeutically effective doses are in the range of 0.2 to 5 mg/kg body weight, inclusive. Still more preferred therapeutically effective doses are in the range of 0.5 to 2 mg/kg, inclusive. Within alternative embodiments, the subsequent therapeutic dose or doses may be in the same or different formulation as the tolerizing dose and/or may be administered by the same or different route as the tolerizing dose.

For the purposes of this invention, the methods of administration are chosen depending on the condition being treated, the form of the subject antibodies, and the pharmaceutical composition.

- 25 Administration of the antibody compositions can be done in a variety of ways, including, but not limited to, continuously, subcutaneously, intravenously, orally, topically, transdermal, intraperitoneal, intramuscularly, and intravesically. For example, microparticle, microsphere, and microencapsulate formulations are useful for oral, intramuscular, or subcutaneous administrations. Liposomes and nanoparticles are additionally suitable for intravenous administrations. Administration of the pharmaceutical compositions may be through a single

20.

route or concurrently by several routes. For instance, intraperitoneal administration can be accompanied by intravenous injections. Preferably the therapeutic doses are administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

5 The compositions may be administered once or several times. In some embodiments, the compositions may be administered once per day, a few or several times per day, or even multiple times per day, depending upon, among other things, the indication being treated and the judgement of the prescribing physician.

Administration of the compositions may also be achieved through sustained release or long-term delivery methods, which are well known to those skilled in the art. By "sustained release or" 10 "long term release" as used herein is meant that the delivery system administers a pharmaceutically therapeutic amount of subject compounds for more than a day, preferably more than a week, and most preferable at least about 30 days to 60 days, or longer. Long term release systems may comprise implantable solids or gels containing the antibodies, such as biodegradable polymers described above; pumps, including peristaltic pumps and fluorocarbon 15 propellant pumps; osmotic and mini-osmotic pumps; and the like.

The method of the invention contemplates the administration of single monoclonal antibodies and any antibody that recognizes the particular antigens recognized by these antibodies, as well as combinations, of different mAbs. Two or more monoclonal antibodies may provide an improved effect compared to a single antibody. Alternatively, a combination of an antibody with 20 an antibody that binds a different antigen may provide an improved effect compared to a single antibody. Such mAb cocktails may have certain advantages inasmuch as they contain mAbs, which exploit different effector mechanisms or combine directly cytotoxic mAbs with mAbs that rely on immune effector functionality. Such mAbs in combination may exhibit synergistic therapeutic effects.

25 In another embodiment, the present invention provides an antibody which binds P2X7 wherein the antibody competes for binding to P2X7 with antibody BPM09 produced by the hybridoma AB253 deposited with the European Collection of Cell Cultures (ECACC) under Accession No. 06080101.

21.

Antibodies which compete for binding with the anti-P2X₇ antibody BPM09 to an antigen may be readily identified using routine competition binding assays known to those skilled in the art, such as the competitive ELISA immunoassay.

5 It is preferred that the level of competition is at least 50%, preferably at least 60% more preferably at least 70%. It is even more preferred that the level of competition is at least 80%, preferably at least 90% preferably at least 95%. It is most preferred that the level of competition is substantially 100%.

The term “% competition” refers to the decrease in binding which occurs in the presence of the competing antibody.

10 The following protocols are provided as non-limiting examples for the purpose of illustrating the invention.

EXAMPLE 1

Production of monoclonal antibodies

15 The preferred animal system for generating hybridomas is the murine system. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are well known in the art. Fusion cell partners (e.g., murine myeloma cell lines SP2/0, NS0, NS1, rat myeloma Y3, rabbit myeloma 240E 1, human K6H6), fusion and screening procedures are also well known in the art (Galfre et al., 1977; Gefter et al., 1977; Galfre et al., 1979; dangl et al., 1982; Spieker-Polet et al., 1995).

20 (i) Hybridoma generation

B cell-myeloma cell hybridomas were generated using splenocytes from immunised mice as follows:

Immunization: BALB/c mice (female, 8-10 weeks of age at first injection, CSIRO animal facility, North Ryde, Australia) were immunized with conjugate comprising human P2X₇ 200-
25 216 linked to diphtheria toxoid (at a conjugation ratio of approximately 11:1) emulsified in

22.

adjuvant. The initial immunization was performed with conjugate in Montanide-QuilA-DEAE dextran, 4 x 50 µg/mL. Subsequent immunizations were performed at 2-4 weekly intervals with conjugate in ImmunEasy™ adjuvant (Qiagen), 2 x 50 µg/mL injections per mouse (1 intramuscularly and 1 subcutaneously), 200 µg/mL. After at least 3 immunization cycles, mice
5 were injected with 20µg conjugate intravenously in sterile phosphate-buffered saline.

Hybridoma generation: Four to five days after the intravenous boost, spleens were recovered and spleen cell suspensions prepared. Spleen cells were fused with SP2/0Ag14 myeloma cells by mixing at a ratio of 5:1 in a 50% solution of polyethylene glycol 1500 (Roche Cat No. 783 641) in serum-free medium (RPMI with 2Mm L-glutamine, 1mM sodium pyruvate, 50IU/mL
10 penicillin and 50µg/mL streptomycin; Gibco). After incubation at 37°C for 2 minutes, the cell suspension was diluted in serum-free medium, and pelleted by centrifugation (8 minutes, 70 x g). Cells were cultivated in RPMI medium (as above, supplemented with 10% foetal bovine serum, HAT (Gibco) and 100U/mL recombinant murine IL-6 (PeproTech). After 12-14 days, cell culture supernatants were assayed for reactivity by ELISA with solid-phase human P2X₇ 200-216
15 linked to bovine serum albumin (at a conjugation ratio of approximately 11:1). Positive wells were subcloned at limiting dilution.

From these positive wells the hybridoma producing antibody AB253 was isolated.

EXAMPLE 2

BPM09 is distinguished from other tumour-selective P2X₇ antibodies in that it results in superior
20 immunohistochemical staining: strong staining of tumour tissue, with minimal staining of normal tissue.

Immunohistochemical protocol/results for AB253

Preparation of tissue sections

1. Sections (3µm) from tumour blocks were floated onto 3-aminopropyltriethoxysilane
25 (AES) coated heat resistant glass slides.

23.

2. Slides were simultaneously dewaxed, endogenous peroxidase-blocked and heat induced epitope retrieval performed (Biocare Medical Universal Decloaker solution) and slides were stained in a Sequenza apparatus.

Staining of tissue sections with AB253

- 5 3. After rinsing with tris-buffered saline (Biocare Medical TBS automation wash buffer), sections were incubated with 80 pM AB253 for 60 minutes. After rinsing twice in TBS, Mach 4 Universal polymer (Biocare Medical) detection system was used, consisting of 15 minute incubation in anti-mouse IgG reagent, followed by rinsing once in TBS and incubation for 25 minutes in HRP-polymer and additional rinsing in TBS (x2).
- 10 4. After stringent washing and DAB (Dako) staining, BPM09-labeled slides were counter stained with haematoxylin (Mayer's with Lillie's modification, from Dako) for 1 minute, rinsed thoroughly and cover-slipped.
5. Photomicrographs were obtained using an Olympus BX41 with 10x objective (Olympus) and a Q Imaging Micropublisher 5 megapixel microscope camera.
- 15 Figure 1 illustrates the binding of BPM09 to human prostate cancer tissue. In comparison, the amount of BPM09 antibody binding to normal tissue was minimal (data not shown), accounting for non-specific antibody binding only.

EXAMPLE 3

Anti-P2X₇ competition ELISA

20 **Materials**

BSA-P2X₇ 200-216 PEPTIDE SEQUENCE (H-GHNYTTRNILPGLNITC-NH₂ conjugated to Bovine Serum Albumin with Maleimidocaproyl-N-Hydroxysuccinimide (MCS) as the linker)

96 well ELISA plates

24.

BSA (Albumin, Bovine Fraction V, Sigma Cat # A-9647)

Tween 20 (Polyoxyethylene-20-Sorbitan Monolaurate, Amresco Cat # 0777-IL)

5 Biotinylated BPM09 (Biotinylation was performed on 1 mg of protein A-purified BPM09 with a 20-fold molar excess of NHS-LC-biotin Pierce Cat 3 21435 according to the manufacturer's instructions).

Secondary conjugate (Streptavidin conjugated to horseradish peroxidase (HRP), Zymed Cat# 434323)

Na_2CO_3 , NaHCO_3 , Na_2HPO_4 , NaH_2PO_4 , NaCl , citric acid monohydrate, tri-sodium citrate dihydrate, H_2O_2

10 ABTS (2,2'-Azino-bis(3-Ethylbenz-Thiazoline-6-Sulfonic acid) Diammonium Salt, Sigma Cat # A-1888)

ELISA plate reader

Solutions

Carbonate Coating Buffer pH 9.6

15 1.59 g of Na_2CO_3 and 2.93 g NaHCO_3 was made up to IL with distilled H_2O , after adjustment to pH 9.6.

0.1M PBS pH 7.2 (stock solution)

68.4 mL of a 1M Na_2HPO_4 solution, 31.6 mL of a 1M NaH_2PO_4 solution and 88 g of NaCl was made up to 1L with distilled H_2O after adjustment to pH 7.2.

20 *0.01M PBS pH 7.2 (working concentration)*

The 0.1M PBS solution was diluted (1/10) with distilled H_2O

25.

Blocking Buffer

1% (w/v) BSA was added to 0.01M PBS pH 7.2

Antibody Diluent

1% (w/v) BSA and 0.05% (v/v) Tween 20 was added to 0.01M PBS pH 7.2

5 *Wash Buffer*

0.05% (v/v) Tween 20 was added to 0.01M PBS pH 7.2

Citrate Buffer (5x) (Stock concentration)

21g of citric acid monohydrate and 14.7g tri-sodium citrate dehydrate was made up 150mL with dH₂O after adjustment to pH to 4.4.

10 *Substrate Solution*

2.4mL of Citrate buffer (5x), 240 μ L ABTS solution [25 mg/mL in H₂O (50x)], 12 μ L of H₂O₂, and made up to 12 mL with distilled H₂O.

Procedure

- 15 1. BSA-₇ was diluted to 3 μ g/mL in Carbonate Coating Buffer, 100 μ L added to each well of a 96 well plate, and incubated at 4°C overnight in a humidified container.
2. The plate was washed in Wash Buffer (3x), then PBS (3x), and blotted onto a paper towel to remove leftover buffer.
3. The plate was blocked with 200 μ L of Blocking buffer at room temperature, in a humidified container for a minimum of 1 hour.
- 20 4. Step 3 was repeated to wash the plate.

26.

5. In a fresh 96 well plate, non-biotinylated BPM09, or an irrelevant specificity isotype-matched control was serially diluted in antibody diluent, and biotinylated BPM09 added (*0.63µg/mL, final conc.).

* biotinylated BPM09 concentration (0.63µg/mL) was determined to give maximum binding in a preliminary ELISA conducted according to this protocol, with the omission of a competitor.

6. The antibody mixture (100 µL) was added to triplicate wells. Wells were included with no peptide coating to determine the background absorbance.

7. After 30 mins incubation at room temperature in a humidified container the plate was again washed according to step 3.

8. Streptavidin-HRP \$1:2000) in antibody diluent (100 µL) was added to each well and incubated at room temperature in a humidified container with gentle mixing for 1 hour.

9. The plate was washed as in step 3 and the freshly prepared substrate solution (100 µL) was added to each well.

10. The colour reaction was allowed to develop for 15 min prior to measurement of the OD at 405nm (ref. 620nm) with an ELISA plate reader.

The results of the ELISA competition binding assays are indicated in Figure 2. Figure 2(A) shows that as the concentration of the non-biotinylated BPM09 antibody is increased, the overall binding of biotinylated BPM09 to the P2X₇ receptor sites decreases. Conversely, Figure 2(B) shows the same competition binding assay performed using an immunoglobulin isotype-matched control where no effect on the binding of biotinylated antibodies is observed. These data clearly show the binding specificity of BPM09 antibody for P2X₇.

It will be understood that the invention disclosed and defined in this specification extends to all alternative embodiments of two or more of the individual features mentioned or evident from the

27.

text or drawings. All of these different combinations constitute various alternative aspects of the invention.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to
5 be considered in all respects and not restrictive.

28.

CLAIMS

1. A hybridoma for producing an antibody that is capable of forming an immune complex with a non functional P2X7 receptor, the hybridoma being characterised in that it produces an antibody that has an affinity for a non functional P2X7 receptor expressed on a live cell of less
5 than about $5 \times 10^6 \text{ M}^{-1}$.
2. The hybridoma according to claim 1 wherein the affinity is about $5 \times 10^5 \text{ M}^{-1}$ or less.
3. The hybridoma according to claim 1 wherein the affinity is about $5 \times 10^4 \text{ M}^{-1}$ or less.
4. The hybridoma AB253 deposited with the European Collection of Cell Cultures (ECACC) under Accession no. 06080101.
- 10 5. An antibody produced by a hybridoma according to any one of the preceding claims.
6. The antibody according to claim 5 wherein the antibody is BPM09.
7. The antibody according to any one of the preceding claims wherein the antibody binds to non functional P2X7 receptors but does not bind to functional P2X7 receptors.
8. A fragment of an antibody according to any one of the preceding claims.
- 15 9. An immune complex formed from the binding of an antibody or fragment according to any one of the preceding claims to a non functional P2X7 receptor, monomer or fragment thereof.
10. The immune complex of claim 9 wherein the non functional P2X7 receptor, monomer or fragment thereof has an amino acid sequence shown in any one of SEQ ID Nos: 1 to 8.
- 20 11. A method for determining whether a cell, tissue or extra cellular body fluid includes a non functional P2X7 receptor, monomer or fragment thereof including:

29.

- contacting a cell, tissue or extra cellular body fluid with an antibody or fragment according to any one of the preceding claims in conditions for forming an immune complex, and

- detecting whether an immune complex has been formed,

wherein detection of an immune complex determines that a cell, tissue or extra -cellular body
5 fluid includes a non functional P2X7 receptor, monomer or fragment thereof.

1/2

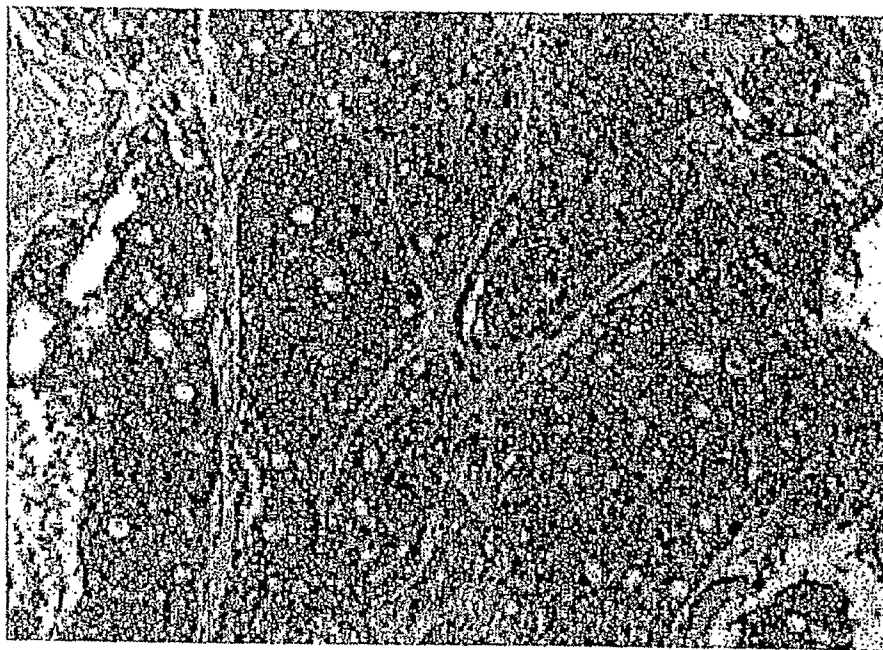


Figure 1

2/2

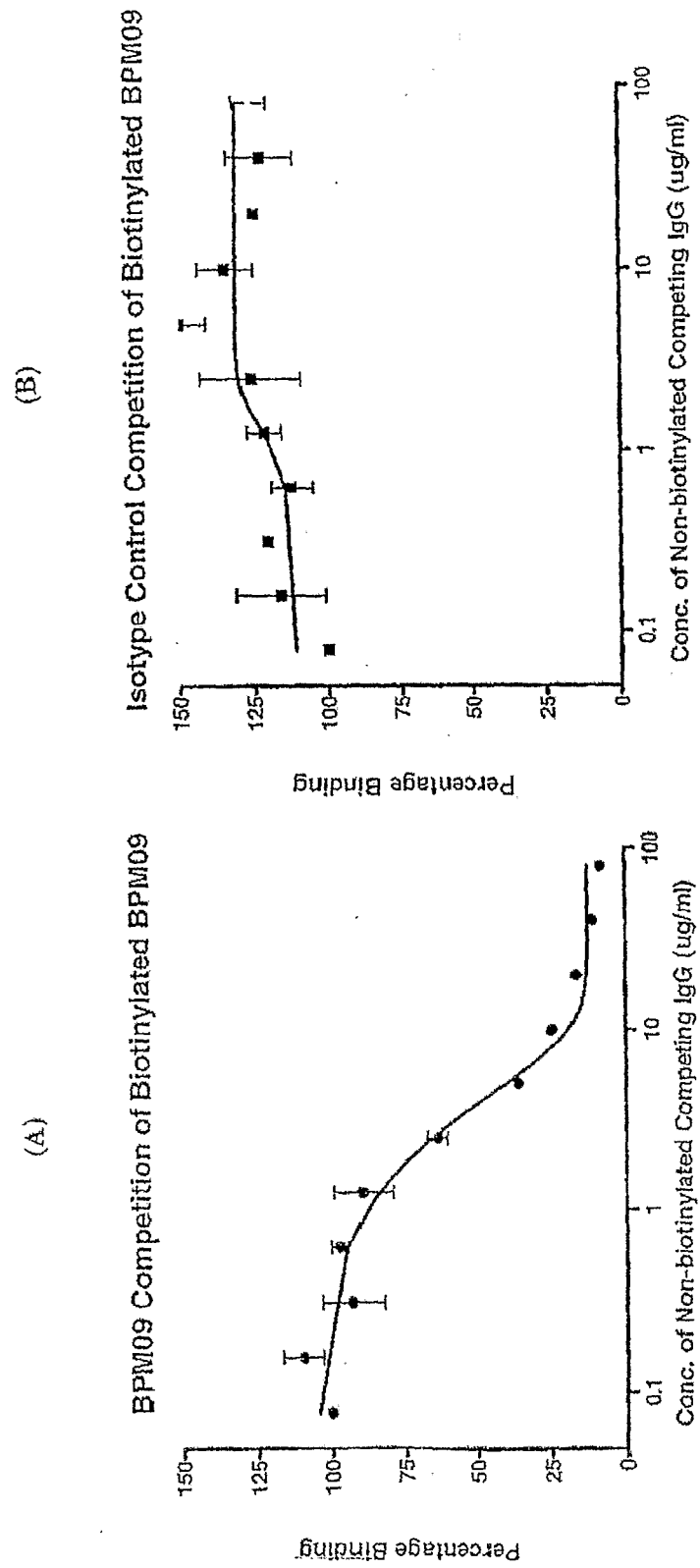


Figure 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2007/001540

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C12N 5/20 (2006.01) *C12N 15/06* (2006.01) *G01N 33/563* (2006.01)
C07K 16/28 (2006.01) *G01N 33/53* (2006.01) *G01N 33/574* (2006.01)
C07K 16/30 (2006.01) *G01N 33/536* (2006.01) *G01N 33/577* (2006.01)

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)

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)

Databases: Chemical Abstracts, WPIDS, Medline, Biosis

Keywords: P2X7, purinergic receptor, antibody, immunoglobulin, non-functional, inactive

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Barden J. A. et al. "Specific detection of non-functional human P2X ₇ receptors in HEK293 cells and B-lymphocytes" FEBS Letters (2003) 538: 159-162 See abstract; page 159, second column, second paragraph; Paragraph headed "2.2. Antibody production"	1-3, 5, 7-11
X	WO 2004/092384 A2 (NEURONOVA AG), 28 October 2004 See Claims 15 and 24; Pages 35-36	1-3, 5, 7-11

☒ 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	"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
"E" earlier application or patent but published on or after the international filing date	"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
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

9 November 2007

Date of mailing of the international search report

7 DEC 2007

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2007/001540

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2003/020762 A1 (INTREAT PTY LIMITED), 13 March 2003 See Claims 1 and 7; Example 4 on page 18	1-3, 5, 7-11
X	WO 2002/057306 A1 (INTREAT PTY LIMITED), 25 July 2002 See page 8 lines 7-16; Page 12 lines 23-27; Examples 1 and 2 on pages 16-17; Claim 30	1-3, 5, 7-11
X	Gu B. J. et al. "An Arg ³⁰⁷ to Gln Polymorphism within the ATP-binding Site Causes Loss of Function of the Human P2X ₇ Receptor" Journal of Biological Chemistry (2004) 279 (30): 31287-31295 See page 31294, first column, last paragraph; Figure 10	1-3, 5, 8-11
X	Wiley J. S. et al. "An Ile-568 to Asn Polymorphism Prevents Normal Trafficking and Function of the Human P2X ₇ Receptor" Journal of Biological Chemistry (2003) 278 (19): 17108-17113 See page 17111, second column, "Expression and Function of Ile-568 to Asn-mutated P2X ₇ "; Figure 4	1-3, 5, 8-11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2007/001540

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: **4 and 6, and dependent claims 5 and 7-11 as far as they relate to Claims 4 and 6**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
There is no technical information in regards to hybridoma AB253 or the antibody BPM09 provided in the description. Specifically, the amino acid sequence of the antibody produced by this hybridoma is not disclosed. Consequently there is no information on which to base a search.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2007/001540

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO 2004092384	CA	2522985	EP	1469072	EP	1473367	
	MX	PA05011008	US	2005147604	US	2006110385	
WO 03020762	AU	2003235005	CA	2434490	CA	2459348	
	CN	1541222	CN	1625565	EP	1360203	
	US	2004142342	WO	02057306	ZA	200402630	
WO 02057306	AU	2003235005	CA	2434490	CA	2459348	
	CN	1541222	CN	1625565	EP	1360203	
	US	2004142342	WO	03020762	ZA	200402630	
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							