~~Combination therapy~~

Use of P2X7 Receptors as Immunogens in Cancer Therapy

Field of the invention

The invention relates to a method of treating or ameliorating diseases that are associated with non-functional $P2X_7$ receptor expression, including cancer.

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: $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_6$, $P2X_7$.

$P2X_7$ 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 $P2X_7$ receptors in normal homeostasis, such as on erythrocytes and in other cell types such as skin at generally lower levels.

Interestingly, a $P2X_7$ receptor containing a monomer having a cis isomerisation at Pro210 (according to SEQ ID NO: 1) and which has compromised ATP binding function at the affected site or sites has been found on cells that are understood to be unable to undergo programmed cell death, such as pre-neoplastic cells and neoplastic cells. This isoform of the receptor has been referred to as a "non-functional" receptor and describes a form of the receptor unable to extend the operating non-selective calcium channel into an apoptotic pore.

Antibodies generated from immunisation with a peptide including Pro210 bind to non-functional $P2X_7$ receptors at the altered ATP binding site/s formed between adjacent monomers. However, they do not bind to $P2X_7$ receptors capable of binding ATP at any of the three available sites. 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 discuss a probe in the form of a monoclonal antibody for distinguishing between functional $P2X_7$ receptors, defined as those receptors able to form a non-selective Ca/Na channel that is additionally able to form an apoptotic pore upon

CLAIMS

1. Use of a $P2X_7$ receptor or fragment thereof in the manufacture of a medicament for minimising the progression of cancer in an individual by forming an immune response in the individual to a P2X7 receptor, wherein the individual has received a non self binding site for the treatment of cancer.

2. Use of claim 1, wherein the individual does not have detectable non self antigen binding sites in circulation.

3. Use of claims 1, wherein the individual does not have detectable cancer.

4. Use of claim 3, wherein the individual has developed an immune response to the non self antigen binding site.

5. Use of claim 1, wherein the individual has received a non self antigen binding site that binds to non-functional $P2X_7$ receptor, but not to functional $P2X_7$ receptor, for treatment of the cancer.

6. Use of any one of the preceding claims, wherein immune response is formed by providing an immunogen in the individual in the form of a $P2X_7$ receptor, or a fragment of a $P2X_7$ receptor that is capable of inducing an immune response to a $P2X_7$ receptor in the individual.

7. Use according to claim 6, wherein the fragment of a $P2X_7$ receptor has an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 3 and 4.

8. Use of claim 6, wherein the non self antigen binding site received by the individual for treatment of the cancer does not bind to a $P2X_7$ receptor, or a fragment of a $P2X_7$ receptor.

9. Use of claim 6, wherein the immunogen is provided in an initial administration to the individual, thereby forming a response that includes IgM production in the individual.

10. Use of claim 6, wherein the immunogen is provided in an initial administration to the individual, thereby forming a response that includes IgM production, and at a later time, in a further administration to the initial administration, thereby forming a response that includes IgG production.

11. Use according to claim 6, wherein the non self antigen binding site or anti-cancer antigen antibody is selected from the group consisting of a dAb, Fab, Fd, Fv, $F(ab')_2$, scFv and CDR.

12. Use of a $P2X_7$ receptor or fragment thereof in the manufacture of a first composition and use of a non self binding site in the manufacture of a second composition, the first and second compositions being used to minimise the progression of cancer in an individual, wherein the first composition is administered to the individual after the second composition.

13. Use of an immunogen in the form of a cancer-associated $P2X_7$ receptor or fragment thereof in the manufacture of a medicament for forming a humoral immune response to cancer-associated $P2X_7$ receptors in an individual who has received an anti-cancer antigen antibody for therapy of cancer,

wherein the immune response is formed in the individual at a time where anti-cancer antigen antibody administered for treatment of cancer is at a level or concentration that is substantially undetectable in the individual; and/or

the humoral immune response to cancer –associated $P2X_7$ receptors is formed according to an immunisation schedule whereby the amount of antibody formed in the individual to cancer-associated $P2X_7$ receptor is about 0.1 to 25 mg/kg individual.

14. Use according to claim 12 or 13, wherein the non self antigen binding site or anti-cancer antigen antibody is selected from the group consisting of a dAb, Fab, Fd, Fv, $F(ab')_2$, scFv and CDR.

15. Use according to claim 14, wherein the fragment of a $P2X_7$ receptor has an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 3 and 4.

16. Use according to claim 1, 12 or 13, wherein the cancer is selected from the group consisting of adenocarcinoma, adenoma, adenofibroma, adenolymphoma, adontoma, AIDS related cancers, acoustic neuroma, acute lymphocytic leukemia, acute myeloid leukemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, ameloblastoma, angiokeratoma, angiolymphoid hyperplasia with eosinophilia, angioma sclerosing, angiomatosis, apudoma, anal cancer, angiosarcoma, aplastic

anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, branchioma, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancers, cutaneous T-cell lymphoma, carcinoma (e.g. Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, Krebs 2, Merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell), carcinosarcoma, cervical dysplasia, cystosarcoma phyllodies, cementoma, chordoma, choristoma, chondrosarcoma, chondroblastoma, craniopharyngioma, cholangioma, cholesteatoma, cylindroma, cystadenocarcinoma, cystadenoma, dermatofibrosarcoma- protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, dysgerminoam, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anaemia, fibroma, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestationaltrophoblastic-disease, glioma, gynaecological cancers, giant cell tumors, ganglioneuroma, glioma, glomangioma, granulosa cell tumor, gynandroblastoma, haematological malignancies, hairy cell leukemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, hamartoma, hemangioendothelioma, hemangioma, hemangiopericytoma, hemangiosarcoma, hemangiosarcoma, histiocytic disorders, histiocytosis malignant, histiocytoma, hepatoma, hidradenoma, hondrosarcoma, immunoproliferative small, opoma, ontraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, langerhan's cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukemia, li-fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leigomyosarcoma, leukemia (e.g. b-cell, mixed cell, null-cell, t-cell, t-cell chronic, htlv-ii-associated, lymphangiosarcoma, lymphocytic acute, lymphocytic chronic, mast-cell and myeloid), leukosarcoma, leydig cell tumor, liposarcoma, leiomyoma, leiomyosarcoma, lymphangioma, lymphangiocytoma, lymphagioma, lymphagiomyoma, lymphangiosarcoma, male breast cancer, malignant- rhabdoid-tumor-of-kidney, medulloblastoma, melanoma,

Merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, malignant carcinoid syndrome carcinoid heart disease, medulloblastoma, meningioma, melanoma, mesenchymoma, mesonephroma, mesothelioma, myoblastoma, myoma, myosarcoma, myxoma, myxosarcoma, nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, Nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer-(nsclc), neurilemmoma, neuroblastoma, neuroepithelioma, neurofibromatosis, neurofibroma, neuroma, neoplasms (e.g. bone, breast, digestive system, colorectal, liver), ocular cancers, oesophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral- neuroectodermal-tumors, pituitary cancer, polycythemia vera, prostate cancer, osteoma, osteosarcoma, ovarian carcinoma, papilloma, paraganglioma, paraganglioma nonchromaffin, pinealoma, plasmacytoma, protooncogene, rare-cancers-and-associated- disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, reticuloendotheliosis, rhabdomyoma, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (sclc), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, sarcoma (e.g. Ewing's experimental, Kaposi's and mast-cell sarcomas), Sertoli cell tumor, synovioma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis-/-ureter), trophoblastic cancer, teratoma, theca cell tumor, thymoma, trophoblastic tumor, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom' s-macroglobulinemia and Wilms' tumor.

17. Use according to claim 1, 12 or 13, wherein the non self antigen binding site or anti-cancer antigen antibody binds to a peptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 3 and 4.

18. Use according to claim 8, wherein the non self antigen binding site binds to any one of the following VEGF, VEGFA, VEGFC, VEGFD, Her-2 or CD20.

19. Use according to claim 1, 12 or 13, wherein when the non self antigen binding site or anti-cancer antigen antibody binds to a $P2X_7$ receptor or fragment thereof, it binds to an

epitope that is different from an epitope or an immunogen that is a $P2X_7$ receptor or fragment thereof used to form an immune response.

20. A composition for use in forming a humoral immune response to cancer-associated in an individual who has received an anti-cancer antigen antibody for therapy of cancer, said composition including an immunogen in the form of a cancer-associated $P2X_7$ receptor or fragment thereof.

21. A kit or article of manufacture including:

an antigen binding site in the form of an immunoglobulin variable domain, antibody, dAb, Fab, Fd, Fv, $F(ab')_2$, scFv or CDR reactive with a $P2X_7$ receptor; and

an immunogen for generating an immune response to a non-functional $P2X_7$ receptor.

22. A kit or article of manufacture according to claim 21, wherein the antigen binding site is reactive with a non-functional P2X7 receptor.

CLAIMS

1. Use of a $P2X_7$ receptor or fragment thereof in the manufacture of a medicament for minimising the progression of cancer in an individual by forming an immune response in the individual to a P2X7 receptor, wherein the individual has received a non self binding site for the treatment of cancer.

2. Use of claim 1, wherein the individual does not have detectable non self antigen binding sites in circulation.

3. Use of claims 1, wherein the individual does not have detectable cancer.

4. Use of claim 3, wherein the individual has developed an immune response to the non self antigen binding site.

5. Use of claim 1, wherein the individual has received a non self antigen binding site that binds to non-functional $P2X_7$ receptor, but not to functional $P2X_7$ receptor, for treatment of the cancer.

6. Use of any one of the preceding claims, wherein the immune response is formed by providing an immunogen in the individual in the form of a $P2X_7$ receptor, or a fragment of a $P2X_7$ receptor that is capable of inducing an immune response to a $P2X_7$ receptor in the individual.

7. Use according to claim 6, wherein the fragment of a $P2X_7$ receptor has an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 3 and 4.

8. Use of claim 6, wherein the non self antigen binding site received by the individual for treatment of the cancer does not bind to a $P2X_7$ receptor, or a fragment of a $P2X_7$ receptor.

9. Use of claim 6, wherein the immunogen is provided in an initial administration to the individual, thereby forming a response that includes IgM production in the individual.

10. Use of claim 6, wherein the immunogen is provided in an initial administration to the individual, thereby forming a response that includes IgM production, and at a later time, in a further administration to the initial administration, thereby forming a response that includes IgG production.

11. Use according to claim 6, wherein the non self antigen binding site or anti-cancer antigen antibody is selected from the group consisting of a dAb, Fab, Fd, Fv, $F(ab')_2$, scFv and CDR.

12. Use of a $P2X_7$ receptor or fragment thereof in the manufacture of a first composition and use of a non self antigen binding site in the manufacture of a second composition, the first and second compositions for minimising the progression of cancer in an individual, wherein the first composition is adapted to be administered to the individual after the second composition.

13. Use of an immunogen in the form of a cancer-associated $P2X_7$ receptor or fragment thereof in the manufacture of a medicament for forming a humoral immune response to cancer-associated $P2X_7$ receptors in an individual who has received an anti-cancer antigen antibody for therapy of cancer,

wherein the immune response is formed in the individual at a time where anti-cancer antigen antibody administered for treatment of cancer is at a level or concentration that is substantially undetectable in the individual; and/or

the humoral immune response to cancer –associated $P2X_7$ receptors is formed according to an immunisation schedule whereby the amount of antibody formed in the individual to cancer-associated $P2X_7$ receptor is about 0.1 to 25 mg/kg individual.

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16. Use according to claim 1, 12 or 13, wherein the cancer is selected from the group consisting of adenocarcinoma, adenoma, adenofibroma, adenolymphoma, adontoma, AIDS related cancers, acoustic neuroma, acute lymphocytic leukemia, acute myeloid leukemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, ameloblastoma, angiokeratoma, angiolymphoid hyperplasia with eosinophilia, angioma sclerosing, angiomatosis, apudoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, branchioma, CNS

tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancers, cutaneous T-cell lymphoma, carcinoma (e.g. Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, Krebs 2, Merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell), carcinosarcoma, cervical dysplasia, cystosarcoma phyllodies, cementoma, chordoma, choristoma, chondrosarcoma, chondroblastoma, craniopharyngioma, cholangioma, cholesteatoma, cylindroma, cystadenocarcinoma, cystadenoma, dermatofibrosarcoma- protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, dysgerminoam, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anaemia, fibroma, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestationaltrophoblastic-disease, glioma, gynaecological cancers, giant cell tumors, ganglioneuroma, glioma, glomangioma, granulosa cell tumor, gynandroblastoma, haematological malignancies, hairy cell leukemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, hamartoma, hemangioendothelioma, hemangioma, hemangiopericytoma, hemangiosarcoma, hemangiosarcoma, histiocytic disorders, histiocytosis malignant, histiocytoma, hepatoma, hidradenoma, hondrosarcoma, immunoproliferative small, opoma, ontraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, langerhan's cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukemia, li-fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leigomyosarcoma, leukemia (e.g. b-cell, mixed cell, null-cell, t-cell, t-cell chronic, htlv-ii-associated, lymphangiosarcoma, lymphocytic acute, lymphocytic chronic, mast-cell and myeloid), leukosarcoma, leydig cell tumor, liposarcoma, leiomyoma, leiomyosarcoma, lymphangioma, lymphangiocytoma, lymphagioma, lymphagiomyoma, lymphangiosarcoma, male breast cancer, malignant- rhabdoid-tumor-of-kidney, medulloblastoma, melanoma, Merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, malignant carcinoid syndrome carcinoid heart disease, medulloblastoma, meningioma, melanoma, mesenchymoma, mesonephroma, mesothelioma, myoblastoma, myoma,

myosarcoma, myxoma, myxosarcoma, nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, Nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer-(nsclc), neurilemmoma, neuroblastoma, neuroepithelioma, neurofibromatosis, neurofibroma, neuroma, neoplasms (e.g. bone, breast, digestive system, colorectal, liver), ocular cancers, oesophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral- neuroectodermal-tumors, pituitary cancer, polycythemia vera, prostate cancer, osteoma, osteosarcoma, ovarian carcinoma, papilloma, paraganglioma, paraganglioma nonchromaffin, pinealoma, plasmacytoma, protooncogene, rare-cancers-and-associated- disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, reticuloendotheliosis, rhabdomyoma, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (sclc), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, sarcoma (e.g. Ewing's experimental, Kaposi's and mast-cell sarcomas), Sertoli cell tumor, synovioma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis-/-ureter), trophoblastic cancer, teratoma, theca cell tumor, thymoma, trophoblastic tumor, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom' s-macroglobulinemia and Wilms' tumor.

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18. Use according to claim 8, wherein the non self antigen binding site binds to any one of the following VEGF, VEGFA, VEGFC, VEGFD, Her-2 or CD20.

19. Use according to claim 1, 12 or 13, wherein when the non self antigen binding site or anti-cancer antigen antibody binds to a $P2X_7$ receptor or fragment thereof, it binds to an epitope that is different from an epitope or an immunogen that is a $P2X_7$ receptor or fragment thereof used to form an immune response.