Abstract: The present invention provides a composition for the treatment of a tumour, comprising: (i) allogeneic or xenogeneic tumour cells; and (ii) a pharmaceutically acceptable excipient. If two or more heterozygous individuals have the same cancer/tumour of the same or similar histological grade, then transplantation of tumour/cancer tissue/cells from one individual to another will not only induce rejection of the transplanted tissue/cancer, but will also increase the immunological awareness of the immune system to peptides shared between the tumours/cancers and other tumours possessing similar peptides.
TUMOUR VACCINE COMPRISING ALLOGENEIC OR XENOGENEIC TUMOUR CELLS

Field of the Invention
This invention relates to the treatment of tumours. In particular, this invention relates to the immune-mediated treatment of tumours.

Background of the Invention
The immune system consists of two branches, that while separated by speed and specificity, are intricately linked, creating a rapid and directed response against both endogenous and exogenous insults. The innate immune system provides immediate host defence against physical, chemical and microbiological insults. It involves neutrophils, monocytes, macrophages, complement, cytokines and acute phase proteins. Despite the lack of antigen specificity, the innate immune system is able to recognize self from non-self, foreign peptides. Adaptive immunity, however, involves B- and T-lymphocytes in a highly specific antigen directed response. One advantage of adaptive immunity is the potential for immune memory, leading to stronger and more rapid response on further stimulation.

The defining feature of adaptive immunity (specific immunity) is the use of T and B-lymphocytes bearing antigen-specific receptors in a targeted immune response. The major T-cells effectors are the T-helper cells, bearing CD4 receptors and cytotoxic T-cells bearing CD8 receptors. T-helper cells interact with MHC I and are responsible for coordinating the immune response, recognizing foreign antigens, activating various parts of the immune system and activating B-cells. Cytotoxic T-cells interact with MHC I receptors and play a role in mounting an immune response against exogenous pathogens.

The Major Histocompatibility Complex (MHC) is a genetic region that codes for proteins that play an essential role in regulating and modulating immune response. The gene products of the MHC are divided into two separate groups, based on structure and biological properties: MHC I and MHC II. MHC class I receptors are present on all nucleated cells. They present endogenously-synthesized peptides and are intimately involved in self-self recognition. MHC class II receptors are only found on cells involved with immune responses and present exogenously-derived proteins such as those from bacterial production. Classically, MHC I was thought to be involved in tumour rejection, but more recently MHC II has been found to play a role.
The diversity of antigen binding by MHC class I molecules is based on three basic and interrelated principles. First, class I molecules have the ability to bind peptides with many different sequences. These MHC class I antigen complexes can be recognized by the cytotoxic T-lymphocytes (CD8), eventually leading to the destruction of any cell carrying a similar foreign protein. Secondly, each organism expresses a number of different class I genes. Lastly, MHC exhibits polymorphisms with a number of alleles at each locus. In humans, the MHC I is represented at more than one locus called the Human Leukocyte Antigen (HLA), the loci being HLA-A, -B and -C, the most polymorphic of which is HLA-B. These factors imply a high degree of individual specificity and the need for a regulator to exert selective pressure on the cellular immune system.

It is this high degree of specificity and overriding selective pressure that is exploited in the transplantation of organs and tissues between individuals. Identical twins and genetically close family members are less likely to reject transplanted tissue since they have similar HLA loci. This is based on the fact that the MHC I are expressed co-dominantly and in most cases inherited intact without recombination. Therefore, homozygous individuals such as identical twins and syngeneic rats could theoretically accept a brain tumour from his/her homozygous donor. Yet more critically, they would reject a brain tumour from a heterozygous donor based on a specific and targeted immune response.

As stated earlier, MHC class I receptors are surface glycoproteins located on most cells that play a crucial role in immune response. These MHC class I bind to antigenic peptides and interact with NK cells and CD8. These peptides are derived from degraded endogenous proteins from virus and tumour infected cells. Antigen processing is a complex mechanism that involves numerous steps. A defect in any of these steps may lead to non-expression of the MHC class I antigen complex and escape from T-cell recognition and destruction. The loss of or dysregulation of MHC I complexes is a frequent mechanism for evading destruction from CD8. Intuitively, one might assume that this "missing self" marker might lead to increased recognition by NK cells, which are inhibited by interacting with the MHC I complex, and stimulated by cells with down-regulated HLA-2/HA expression. Yet even with a fully functioning immune system, it is possible for tumours to evade recognition through the use of an elusive escape strategy. Although the mechanism of escape is poorly understood, experiments have described several mechanisms allowing tumours to escape recognition by the immune system. These mechanisms range from loss or
mutatso π o f HLA hatotypes t o unresponsiveness t o interferons 12 So while a change of or loss of MHC class I receptors is associated with the genesis of various tumours, the presence of MHC class I molecules has been shown to participate in cancer resistance

An example of the anti-tumourogenic effects of MHC class I molecule 1 is in the immune surveillance of mitochondrial DNA integrity In one study, one of the roles of MHC I molecules was to eliminate cells carrying mitochondrial mutation 13 Human glioma cells carry multiple mutations in both the mitochondrial DNA and in the mitochondrial complex 7 From this data, it is possible to assume that gliomas of the same histological type/grade will carry similar mutations in their DNA and have similar abnormal surface proteins associated with both MHC class I molecules and the cell membrane Conversely, an intact immune system can also allow for the development and progression of tumours

It has been shown that the progression of certain cancers is associated with the expression of tumour-specific antigens and an associated immune response 15 Therefore, effective tumour rejection and immunity cannot be achieved solely by self-vaccination Despite these barriers, there is increasing evidence that the immune system can be used to combat cancer While both a dysregulated and normally functioning immune system fight against immune rejection of cancer, there have been reported results of the spontaneous rejection of malignant tumours 1926 Interestingly, it has also been suggested that autoimmune diseases may contribute to a better prognosis in patients with malignant tumours 6 19 In these patients, the majority of the IgG specificities identified share considerable homology with both human and microbial peptides 14 This has led to the hypothesis that molecular mimicry may initiate the observed tumour autoimmunity Studies related to this have shown long term remission of malignant brain tumours after intracranial infection in four patients 4, and improved survival of cancer patients with microbial infection 2022 This brings into question whether the molecular mimicry induced autoimmunity can be used to treat tumours Importantly, significant homology has been shown to exist between human proteins and proteins from other species 24 Further experiments have shown that xenogeneic antigen from endothelial cells can break immune tolerance against autologous angiosgenic endothelial cells 20 This suggests that self-tolerance to tumours may be broken through cross-reactivity with a homologous foreign antigen

Summary of the Invention
The present invention is based on the realisation that, if two or more heterozygous individuals have the same cancer/tumour of a similar or same histological grade, then transplantation of tumour/cancer tissues/cells from one individual (or more) to another (or others) will not only induce rejection of the transplanted tissue/cancer but will also increase the immunological awareness of the immune system to peptides shared between the tumours/cancers and other tumours possessing similar peptides. Allogeneic tumours may therefore be used to vaccinate an established tumour, reduce its size or eliminate it and establish lasting memory.

This technique will not only lead to eventual rejection of the primary tumour, but will also lead to a lasting immunologic memory, preventing the organism from developing the tumour again.

According to a first aspect of the invention, a composition for the treatment or prevention of a tumour comprises:

(a) allogeneic or xenogeneic tumour cells, and

(ii) a pharmaceutically acceptable excipient

The allogeneic or xenogeneic cells may be provided from one or more allogeneic or syngeneic individuals and presented either as whole cells or as a lysate. The composition may also comprise a lysate of a syngeneic cell.

According to a second aspect of the invention there is the use of allogeneic or xenogeneic tumour cells in the manufacture of a medicament for the treatment of a tumour in a patient.

The medicament may further comprise a lysate of a syngeneic cell. The allogeneic or xenogeneic tumour cells may be a whole cell or may be a lysate.

The present invention not only treats tumours but has the benefit that the immune system is able to effectively target the tumour, avoiding problems associated with delivery/targeting of conventional chemotherapeutics. In addition, chemotherapy has many undesirable side-effects including baldness, nausea, diarrhea, anaemia and increased risk of infection, and these are avoided using the therapy of the invention.

Description of the Drawings

The invention is described with reference to the accompanying figures wherein:

Figure 1 is a schematic of experimental design in Sprague Dawley Rats. Rats were divided into two groups. Those receiving syngeneic cell line (C6 SD-A) and those receiving allogeneic cell line (9L SD-B). The syngeneic arm of the study was divided into two groups: control (5 rats SD-A1) and treatment (4 rats SD-A2). On day 27 4...
SD-A1 rats were sacrificed. At this time the remaining SD-A1 rat (Rat 9) entered the treatment protocol. All SD rats initially injected with the 9L allogeneic arm (SD-B) received flank injections with the syngeneic C6 cell line (100,000 cells), followed ten days later with an additional bolus of 500,000 C6 cells.

Figure 2 is a schematic of experimental design in Fisher 344 rats. Rats were divided into two groups. Those receiving syngeneic 9L cell line (Fisher A) and those receiving allogeneic C6 cell line (Fisher B). The rats in the syngeneic group (Fisher A) were divided into a control group (Fisher A-1) and a treated group (Fisher A-2). Fisher A-1 rats were challenged with syngeneic RG2, 9L, or medium only. Fisher A-2 rats were treated with allogeneic cells, allogeneic cells and cell lysate, syngeneic cell lysate and xenogeneic cells, or xenogeneic cells alone. Fisher B rats were initially injected with C6 allogeneic cells. They were subsequently challenged with flank injections of syngeneic 9L cells (100,000 cells) followed ten days later with an additional bolus of 500,000 cells.

Figure 3 is a graph charting tumour evolution/progression in 9 SD rats with subcutaneously implanted syngeneic tumour (C6). Rats were placed in either control or treatment groups as previously described. Tumour progression was determined through measurements of tumour volume (mm³). Rat 1,2,3,4 received no treatment after C6 tumour implantation. Rat 5,6,7,8 received treatment with allogeneic 9L cells and lysates with syngeneic C6 lysate. Rat 9 was allowed to form a very large tumour before it was switched to the treatment group as in Rats 5-8.

Figure 4 is a graph charting tumour evolution/progression in Fisher 344 rats with subcutaneously implanted syngeneic tumour (9L). Control rats (Fisher A-1) were then injected with syngeneic RG2 cells (Rat 1), syngeneic 9L cells (Rat 2), or medium alone (Rat 3). Rat 1 had double the dosage of cells injected, and formed an extremely large tumour. Treated rats (Fisher A-2) were treated with allogeneic C6 cells alone (Rat 4), allogeneic C6 cells and lysate (Rat 5), syngeneic 9L lysate and xenogeneic U87 and LN229 cells (Rat 6), xenogeneic U87 and LN229 cells alone (Rat 7). Rat 8 was treated with syngeneic 9L cell lysate alone. Tumour progression was determined through measurements of tumour volume (mm³).

Figure 5 shows tumour sections taken from A control and B. Treatment Fisher 344 rats. Sections were cut at a thickness of 7 µm and stained with an antibody directed against CD4 receptor according to the previously described protocol. Arrows indicate the location of cells staining positively for the CD4 surface marker. The magnification of both control (A) and treatment (B) sample is 4OX.
Figure 6 shows tumour sections taken from A control and B Treatment Fisher 344 rats. Sections were cut at a thickness of 7 µm and stained with an antibody directed against CD8 receptor according to the previously described protocol. Arrows indicate the location of cells staining positively for the CD8 surface marker. The magnification of both control (A) and treatment (B) sample is 40X.

Figure 7 shows tumour sections taken from A control and B Treatment Fisher 344 rats. Sections were cut at a thickness of 7 µm and stained with an antibody directed against B-lymphocytes (CD 20) according to the previously described protocol. Arrows indicate the location of cells staining positively for the CD 20 surface marker (Figure 5b). The magnification of both control (A) and treatment (B) sample is 40X.

Figure 8 shows tumour sections taken from A control and B Treatment Fisher 344 rats. Sections were cut at a thickness of 7 µm and stained with an antibody directed against macrophages (CD 68) according to the previously described protocol. Arrows indicate the location of cells staining positively for the CD 68 surface marker.

The magnification of both control (A) and treatment (B) sample is 40X.

Figure 9 shows tumour sections taken from A control and B Treatment Fisher 344 rats. Sections were cut at a thickness of 7 µm and stained with an antibody directed against Dendritic cell marker (DRC) according to the previously described protocol. Arrows indicate the location of cells staining positively for the DRC surface marker.

The magnification of both control (A) and treatment (B) sample is 40X.

Description of the Invention

The present invention utilises allogeneic or xenogeneic tumour cells and syngeneic cells, to increase the awareness of a patient's immune system, to treat a tumour. The tumour cell vaccines of the present invention share similar peptides in the cancer/tumour with the same histological grade, with different MHC Smolecules.

The vaccines are prepared with whole allogeneic or xenogeneic tumour cells or lysates of allogeneic or xenogeneic tumour cells. The vaccine may further comprise a lysate of one or more syngeneic cells.

The allogeneic or xenogeneic cells will preferably be tumour cells of the same (or similar) histological grade as the tumour cells of the patient to be treated. Accordingly, if the patient has a brain glioblastoma, the vaccines will be prepared with allogeneic or xenogeneic brain glioblastoma cells. In this way, the peptides comprised in the cells will be similar and will increase the likelihood of the appropriate immune response being generated.
The vaccine compositions will usually comprise allogeneic or xenogeneic cells/lysates from two or more heterozygous individuals. Preferably, the vaccine compositions are prepared using allogeneic or xenogeneic cells/lysates from at least three heterozygous individuals.

The term "allogeneic" refers to cells taken from different individuals of the same species.

The term "xenogeneic" means that which is derived or obtained from an organism of a different species.

The term "syngeneic" refers to genetically identical members of the same species. For example identical twins will have cells and tissues that are syngeneic.

Reference is made in the description to cancers/tumours of the "same or similar histological grade". The skilled person will understand that this is refers to cancers of the same type and which exhibit the same level of differentiation. Grading may be carried out according to the Elston-Ellis method (Simpson et al, J. Clin. Oncol., 2000; 18:2059-2069).

The reference to "cancer" and "tumour" are used interchangeably.

The present invention provides a pharmaceutical composition comprising administering a therapeutically effective amount of allogeneic or xenogeneic tumour cells and optionally pharmaceutically acceptable excipients.

The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine and will typically comprise a pharmaceutically acceptable excipient. Acceptable excipients for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). The choice of pharmaceutical excipient can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the excipient any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s). The reference to "excipient" includes diiuenents and carriers.

Preservatives, stabilizers and dyes may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

There may be different composition/formulation requirements dependent on the different delivery systems.
Where appropriate, the pharmaceutical compositions can be administered by injection, parenterally, for example intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood.

Vaccines may be prepared from the compositions of the invention.

The preparation of vaccines which contain immunogenic cells as active ingredients, is known to one skilled in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified, or the cells encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof.

In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-tosigutamyln-L-alan ine-2-(1'2:..dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylam ine (CGP 19835A, referred to as lviTP-PE), and Ribi, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion.

Further examples of adjuvants and other agents include aluminum hydroxide, aluminum phosphate, aluminum potassium sulfate (alum), beryllium sulfate, silica kaolín carbon, water-in-oil emulsions, oil-in-water emulsions, muramyl dipeptide, bacterial endotoxin, lipid X, Corynebacterium parvum (Propionobacterium acnes), Bordetella pertussis, polyribonucleotides, sodium alginate, lanolin, lysolecithin vitamin A, saponin liposomes i.evarmolsoie, DEAB-dextran, blocked copolymers or other synthetic adjuvants. Such adjuvants are available commercially from various sources, for example, Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.) or Freuni's incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Michigan).
Typically, adjuvants such as Amphigen (oil-m-water), Alhydrogel (aluminum hydroxide), or a mixture of Amphigen and Alhydrogel are used. Only aluminum hydroxide is approved for human use.

The proportion of immunogen and adjuvant can be varied over a broad range so long as both are present in effective amounts. For example, aluminum hydroxide can be present in an amount of about 0.5% of the vaccine mixture (Al2O3 basis). Conveniently, the vaccines are formulated to contain a final concentration of immunogen in the range of from 0.2 to 200 mg/ml, preferably 5 to 50 mg/ml, most preferably 15 mg/ml.

The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly.

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient. The dosages below are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited.

The amounts of each component will be apparent to the skilled person. Suitable amounts are as follows:

- a) 1 x 10⁶ (± 0.5 x 10⁵) allogeneic tumour cells with different H.I.H.C I modified by DNP
- b) 2 x 10⁶ (± 1 x 10⁵) autologous tumour cells modified by DNP and irradiated
- c) 2 x 10⁵ (± 1 x 10⁶) lysate cells of autologous (syngeneic) tumour
- d) 1 x 10⁵ (± 0.5 x 10⁶) lysate cells of allogeneic tumour

The invention will now be described further with reference to the accompanying figures.

Example

Cell lines and Cell culture

The cell lines used in this experiment were the rat glioma cell lines (9L, C6, RG2) and the human glioma cell lines (U87, LN229). All lines were obtained from the American Type Tissue Collection (ATTC), and grown in Dulbecco's Modified Eagles Medium (DMEM) (GIBCO, Grand Island, NY) supplemented with 10% heat-killed Fetal Calf Serum (FCS) 5% penicillin-streptomycin, and Hepes buffer in a humidified incubator at 37°C in a 5% CO₂ atmosphere.

Cell Lysate Preparation

1 x 10⁵ cells were placed in 5 ml tube in culture medium and centrifuged for 5 mm at 250 x 10³ rpm. The supernatant was discarded and 150 µl of sterile distilled
water was added to the tube. The cell/water solution was mixed well and transferred to a 1.0 mi Eppendorf tube and centrifuged at 1.0 x 10^4 for 10 minutes. The supernatant was not discarded and this entire solution was used for cell lysate injections.

**Antibodies and Immunohistochemistry:**

Tumour samples taken from the Fisher 344 rats were frozen in Optimum Temperature Compound (OTC) and cut into 7 µm sections on a cryostat. These sections were dried, fixed with acetone, and washed well with PBS for 1-2 minutes. Blocking was done using the immune serum from the species the secondary antibody was taken from. Slides were washed thoroughly again and then stained with primary antibody against CD-4, CD-57 (Nora Castro Lab Ltd., Burlingame, CA). CD-8, dendritic reticulum cells (DRC) (Dako Corporation, Carpentaria, CA), CD-20, CD-68 (Ventana, Tucson, AZ). Slides were washed again and a secondary biotinylated antibody was added. They were rinsed again and placed in a solution of 3% hydrogen peroxidase and 9 parts 1% sodium azide in PBS. Slides were then rinsed and ABC was added for 30-40 minutes. They were washed with PBS and developed using diaminobezidine tetrahydrochloride and counterstained. Photos of all slides were taken by light microscopy.

**Tumour growth analysis:**

All tumours were found through visual inspection and palpation. Once discovered, the area around the tumour was further exposed by shaving with an electric razor. At the time of injection, tumour size was measured in millimeters using Vernier calipers. Measurements were taken in the cranial/caudal (length), superior/inferior (height), and medial/lateral (width) direction. Tumour volume was calculated by length x width x height x 0.5.

**In-vivo studies:**

All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Southern California. All rats were maintained in a pathogen free environment. For the experiment, we used Sprague Dawtey and Fisher 344 rats. All rats were male and between the ages of 4-6 weeks. Rats were obtained from Harlan (Indianapolis, Indiana). In the subcutaneous tumour model, C6 and 9L were collected using only DMEM to wash them from the tissue culture flasks. Syringes were then prepared containing 100,000-150,000 cells suspended in 150 μl.

Sprague Dawley (SD) rats were divided into two groups (Figure 1). SD-A (9 rats) were implanted with the C6 glioma, a syngeneic ūke glioma eel! line for SD rats.
SD-B (3 rats) were injected with the 9L allogeneic cell line. Once a palpable flank tumour developed in the Group A rats, they were further divided into two groups. SD-A1 (control group-5 rats) received no injections, and SD-A2 (treatment group-4 rats) were injected with a combination of allogeneic 9L cells, allogeneic 9L lysate, and syngeneic C6 lysate. On day 27, 4 of the 5 SD-A1 were sacrificed. At this time, one of the control rats, rat 9, started receiving the same treatment protocol as the SD-A2 rats. The combination of allogeneic cells, syngeneic and allogeneic lysates, was used to enhance the immune response. SD-B rats, which never formed tumours were tested for immune memory by challenging them with syngeneic C6 cells (100,000 cells) and another boost of 500,000 C6 cells 10 days later, and checked for formation of a flank tumour.

Fisher rats were also divided into two groups (Figure 2). Fisher-A (8 rats) were implanted with the syngeneic 9L cell line. Fisher-B (3 rats) were injected with the allogeneic C6 cell line. Once a palpable flank tumour developed in the Fisher-A rats, they were further subdivided into two groups. Fisher A-1 (control group-3 rats) received injections of syngeneic 9L cells, syngeneic RG2 cells, or medium only. Fisher A-2 (treatment group-5 rats) received a combination of allogeneic C6 cells, allogeneic C6 cell lysate, or xenogeneic U87 and LN229 cells (see Figure 2). Fisher B rats, which initially formed tumours that were subsequently rejected, were tested for immune memory by challenging them with a combination cocktail of syngeneic 9L cells and lysate and checked for tumour growth.

Harvesting subcutaneous tissue

All experimental animals were euthanized with an overdose of pentobarbital. All tumours were removed and dissected under sterile conditions, cut into 4 pieces and stored at -80°C. All tumour sections were cut at 7 μm and stained by immunohistochemistry.

Results

Most studies of glioblastoma employ small laboratory animal models. The immune-competent host models most frequently used include two different strains of rat, the Sprague Dawley and the Fisher 344 rats. C6 is a syngeneic-like cell line for the SD rats, while the 9L and RG2 cell lines are syngeneic for the Fisher 344 rats.

Three of the Fisher rats were injected with C6 cell line (Fisher B). While well vascularized tumours did form, they were subsequently rejected in forty days. A similar procedure was used to treat three SD rats with 9L cell line (SD-B). Each of the SD rats rejected the 9L tumour without palpable tumour growth. The SD-A
rats were each injected with the C6 ceų line. All of these rats developed visible
tumours within ten days. At this point, five rats were kept as a control group (SD-A1)
while the remaining four rats were placed into treatment groups (SD-A2). On day 27
rats 1-4 were sacrificed and an attempt was made to "rescue" rat 9. At this time, rat
9 entered the treatment group and started receiving the same injections as the SD-A2
group.

We analysed the tumour growth and treatment response in the nine SD-A rats
(Figure 3). In the treated SD-A2 rats (rats 5-8), individual rats were given different
combinations of allogeneic and syngeneic lysates, as well as cells. For instance, after
five days, rat number 5 had a palpable flank tumour and received one injection
contralateral to the tumour with allogeneic 9L lysates (50,000), syngeneic C6 lysates
(50,000), and 9L allogeneic cells (50,000). Five days after injection, the tumour
resolved. Rats 6,7,8 (SD-A2 rats) all had visible tumours 18 days post injection. At
this time, they each received an injection with 50,000 allogeneic 9L lysate cells plus
50,000 syngeneic C6 lysate cells and 50,000 91 allogeneic cells. These injections
were repeated on days 23 and 28. Rat 6 had an additional treatment at day 33, 15
days after treatment began. The untreated rats (SD-A1, rats 1-4) were sacrificed 27
days post injection due to tumour size. Compared to the tumour progression in the
untreated rats (rats 1-4), rats 5,6,7,8 (SD-A2) had complete resolution of their tumours.

Rat number nine began the experiment in the non-treated group, and then was rescued
days after sacrificing rats 1-4. Rat 9 received injections every four days with allogeneic
9L lysates (50,000) plus C6 syngeneic lysates (50,000) and 9L allogeneic cells
(50,000) and was sacrificed for histological analysis at day 55, when tumour size was
11% from the initial time of rescue.

Twenty days later, the "treated- SD rats with no measurable tumour were
reinjected in the contralateral hind flank with the same syngeneic C6 tumour cell line
that caused the original tumour, using 100,000 cells first and five times as many cells
(500,000 cells) ten days after. The rats were monitored every three days for any sign
of visual or palpable tumour growth. At 160 days, the SD rats remained tumour free.

Figure 4 shows tumour growth and response to the treatment of eight Fisher
rats (Fisher-A) implanted with 9L cells. Rats 1, 2 and 3 (Fisher A-1) received
contralateral flank injections at day 20 with syngeneic RG2 (100,000 cells; rat 1) and
9L (100,000 cells; rat 2), or medium alone (rat 3). There was no decrease in tumour
size, and the RG2 even seemed to have a synergistic effect in rat 1. Rats 4-8 (Fisher
A-2), injected with C6 allogeneic cells/ C6 allogeneic lysate, 9L syngeneic lysate, or U87 and LN229 xenogeneic cells, all appeared to have a reduction in tumour size.

All Fisher 344 rats were sacrificed at day 40 when they developed a foot drop and inability to ambulate. The tumours from each of these rats was removed and cut for staining. We found our treatment tumours to have significantly greater numbers of CD4, CD8, B-lymphocyte (CD20), macrophage (CD68), and dendritic cells than the control tumours (Figures 3-7).

To determine the role of immune memory, we examined whether the SD and Fisher 344 rats initially injected with an allogeneic cell line would be able to accept a syngeneic cell line. The Fisher 344 rats who rejected the C6 (Fisher B) after 40 days were re-injected with syngeneic 9L cells, the SD rats (SD-B) who initially rejected the 9L cell line were injected with C6 cells. All injections were done in the contralateral flank to the original injection and with 500,000 cells. In the SD rat, no visual or palpable tumour developed. In the Fisher, a small (<1 cm x <1 cm x 1 cm) growth developed at the injection site. This growth was noticeable only to palpation, and became progressively smaller and completely undetectable by 10 days. Both strains of rat remained without tumour at 150 days.

These results suggest that the repeated subcutaneous injection of allogeneic (or xenogeneic) cells, allogeneic cell lysates and syngeneic cell lysates lead to the reduction in tumour size by increasing the immunological awareness.

All publications referred to in the description are incorporated herein by reference.
References


10. Duquesnoy Ri, Trucco M Genetic basis of cell surface polymorphisms encoded by the major histocompatibility complex in humans CS Rev Immunol 8 103-145, 1988


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Claims

1. A composition for the treatment or prevention of a tumour, comprising
   (i) allogeneic or xenogeneic tumour cells; and
   (ii) a pharmaceutically acceptable excipient.

2. A composition according to claim 1, further comprising a lysate of a syngeneic cell.

3. A composition according to claim 1 or claim 2 wherein (i) is a lysate.

4. A composition according to any of claims 1 to 3, further comprising a lysate of an allogeneic or xenogeneic cell.

5. A composition according to any preceding claim, wherein the cells of (i) are brain-derived cells.

6. A composition according to claim 5 when pendant on claim 2, wherein the syngeneic cells are brain-derived cells.

7. A composition according to any preceding claim, wherein the allogeneic or xenogeneic cells are derived from the tumours of two or more heterozygous individuals.

8. Use of allogeneic or xenogeneic tumour cells, in the manufacture of a medicament for the treatment of a tumour in a patient.

9. Use according to claim 8, wherein the tumour of the patient is of the same histological grade as the tumour of the allogeneic or xenogeneic cells.

10. Use according to claim 8 or claim 9, wherein the medicament further comprises syngeneic cells or a lysate of syngeneic cells.

11. Use according to any of claims 5 to 7, wherein the allogeneic tumour cells are provided as lysates.

12. Use according to any of claims 8 to 11, wherein the allogeneic or xenogeneic tumour cells are derived from tumours of two or more heterozygous individuals.

13. A method for the treatment or prevention of a tumour, comprising administering to a patient a composition as defined in any of claims 1 to 7.
Sprague Dawley (12 Rats)

Injection of SQ C6 (9 rats) 100% tumor take (SD-A)

Injection of 9L allogeneic cell line (3 rats). No tumors formed (SD-B).

SD-A1
Control Group (5 rats)
No treatment
4 rats sacrificed day 27

SD-A2
Treatment Group (4 rats)

Immune Memory?
100,000 cells C6 + 500,000 cells C6 10 days later.

Check flank for tumor growth/progression

50,000 cells 9L + 50,000 cells 9L lysate + 50,000 C6 Lysate

One control rat, rat 9, shifted to treatment group

Check flank for tumor growth/progression

Fig 1
**Fig 2**

Injection of SQ syngeneic tumor, 9L (8 rats). 100% tumor take (Fisher-A).

Injection of C6 allogeneic cell line (3 rats). SQ tumor Spontaneously rejected (Fisher-B).

Immune Memory?

100,000 cells 9L + 500,000 cells 9L 10 days later.

Check flank for tumor growth/progression

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Rat 1: RG2 cells
Rat 2: 9L cells
Rat 3: Medium only
Syngeneic cells and medium control
Allogeneic cells/Lysates and Syngeneic Lysates

Rat 4: 150,000 C6 cells
Rat 5: 100,000 C6 cells + 100,000 C6 cell lysate
Rat 6: 100,000 cells 9L lysate + 50,000 cells U87 + 50,000 cells LN229
Rat 7: 50,000 cells LN229 + 50,000 cells U87
Xenogeneic Cells

Check flank for tumor growth/progression
TUMOR GROWTH IN SD RATS

Fig 3
TUMOR GROWTH IN FISHER RATS

Fig 4
Figures

5.)
Fig 9
### INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K39/00 A61P35/00

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)

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 practical search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
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<tr>
<th>Category</th>
<th>Citation of document, with indication where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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<tr>
<td>X</td>
<td>WO 90/03183 A (UNIV SOUTHERN CALIFORNIA [US]) 5 April 1 1990 (1990-04-05)</td>
<td>1,3,4,7, 8,10-13</td>
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<td></td>
<td>abstract page 7, line 29 - page 8, line 34 claims 1,13,25-28; examples 2-4</td>
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<td>X</td>
<td>WO 2004/018659 A (LYCOTOPE GMBH [DE]; GOLETZ STEFFEN [DE]; BAUMEISTER HANS [DE]; SCHOEB) 4 March 2004 (2004-03-04)</td>
<td>1-8, 10-13</td>
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<td></td>
<td>page 8, paragraphs 2,3 page 19, last paragraph - page 20, paragraph 2 page 21, paragraph 3 page 23, last paragraph page 25, paragraphs 2,3</td>
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Furtierdocument ments are listed in the continuation of Box C See patent family annex

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

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

"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)

"D" 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

"Y" 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 13 April 2007

Date of mailing of the international search report 27/04/2007

Name and mailing address of the ISA/Authorized officer

Noe, Veerle
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<td>wo 00/33870 A (ONYVAX LTD [GB]; DALGLEISH ANSUS GEORGE [GB]; SMITH PETER MICHAEL [GB]) 15 June 2000 (2000-06-15) abstract page 4, paragraph 3 - page 5, paragraph 4 example 1</td>
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<td>CHAN ARDEN D ET AL: &quot;Active immunotherapy with allogeneic tumor cell vaccines: Present status&quot; SEMINARS IN ONCOLOGY, vol. 25, no. 6, December 1998 (1998-12), pages 611-622, XP008077525 ISSN: 0093-7754 the whole document</td>
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<td>DOLS A ET AL: &quot;ALLOGENEIC BREAST CANCER CELL VACCINES&quot; CLINICAL BREAST CANCER, XX, XX, vol. 3, no. SUPPL 4, February 2003 (2003-02), pages S173-S180, XP009064465 ISSN: 1526-8209 the whole document</td>
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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:
     - Although claim 13 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. **Claims Nos.:**
   - 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:

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).

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 an additional fee, this Authority did not invite payment of any additional fee.**

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.:**
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