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71. **SOLICITANTE** Neotec Pharma PLC

DOMICILIO Gran Bretaña

74. **APODERADO** Dilia María Rodríguez D' Aleman

22. **BOGOTÁ, D. C.,** Enero 30/07

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1) SOLICITUD DE

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2) SOLICITANTE (71)	Nombre: NEUTEC PHARMA PLC Dirección: Central Manchester & Manchester, Children s University Hoispitals NHS Trust, Oxford Road, Manchester M13 9WL, Gran Bretaña Nacionalidad o domicilio Manchester, Gran Bretaña Lugar de constitución Gran Bretaña	IDENTIFICACIÓN C C [ ] NIT [ ] C E [ ] OTRO [ ] Cual ______ NÚMERO ______
	Teléfono Fax E Mail	
3) REPRESENTANTE O APODERADO	Nombre DILIA MARÍA RODRÍGUEZ D ALEMAN Dirección Carrera 11 N° 86-53 Piso 6 Bogotá D C Teléfono 6181088 Fax 6350824 E Mail info@clarkemodet com co	IDENTIFICACIÓN C C [X] NIT [ ] C E [ ] OTRO [ ] NÚMERO 41 654 882 TP 20824 CSJ
4) INVENTOR (ES) (72)	Nombre 1 BURNIE, James, Peter, 2 MATTHEWS, Ruth, Christine, 3 CARTER, Tracey Dirección 1, 2 y 3 Neutec Pharma PLC, Central Manchester & Manchester, Childrean s University Hospitals, NHS Trust, Oxford Road, Manchester M13 9 WL, Gran Bretaña Nacionalidad o Domicilio Todos los anteriores, Gran Bretaña	

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10) ANEXOS

- [x] Comprobante de pago de la tasa de presentación de la solicitud
- [ ] Comprobante de pago por reivindicación de prioridad
- [ ] Documento que acredita la existencia y representación legal cuando el solicitante sea persona Jurídica
- [ ] Poderes si fuera el caso
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- [ ] Declaraciones
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- [ ] Arte final 12 x 12 y 6 x 6 cm
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- [x] Otros

1 Reporte Internacional de búsqueda

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12) Solicitud concesión de la patente,

NOMBRE DILIA RODRÍGUEZ D ALEMAN

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(71) Applicant *(for all designated States except US)*: NEUTEC PHARMA PLC [GB/GB]; Central Manchester & Manchester, Children's University Hospitals NHS Trust, Oxford Road, Manchester M13 9WL (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BURNIE, James, Peter [GB/GB]; NeuTec Pharma plc, Central Manchester & Manchester, Children's University Hospitals NHS Trust, Oxford Road, Manchester M13 9WL (GB). MATTHEWS, Ruth, Christine [GB/GB]; NeuTec Pharma plc, Central Manchester & Manchester, Children's University Hospitals NHS Trust, Oxford Road, Manchester M13 9WL (GB). CARTER, Tracey [GB/GB]; NeuTec Pharma plc, Central Manchester & Manchester, Children's University Hospitals NHS Trust, Oxford Road, Manchester M13 9WL (GB).

(74) Agent: ARENDS, William, Gerrit; Lloyd Wise, McNaight & Lawrence, Commonwealth House, 1-19 New Oxford Street, London WC1A 1LW (GB).

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(54) Title: TREATMENT OF CANCER

(57) Abstract: The present invention relates to a novel medicaments and preparations comprising effective anti-cancer agents together with an anti-Hsp90 antibody which together provide an enhanced efficacy in the treatment of cancer, and leukaemia.

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TREATMENT OF CANCER

The present invention relates to novel medicaments and preparations comprising effective pharmaceutical agents together with an anti-Hsp 90 antibody which together provide an enhanced efficacy in the treatment of cancers, including colorectal cancer. Other aspects of the invention are concerned with the treatment of leukaemias.

CANCER THERAPY AND TREATMENT OF LEUKAEMIA

A first aspect of the present invention relates to novel medicaments and preparations comprising effective anti-cancer agents together with an anti-Hsp90 antibody which together provide an enhanced efficacy in the treatment of cancer.

Members of the heat shock proteins (Hsp) family of proteins have emerged in recent years as having an important role in oncogenesis and cell death. Indeed, heat shock proteins have been identified as being potential targets for cancer therapy for many years (Whitesell L *et al.*, PNAS USA, 1994 Aug 30, 91(18): 8324-8; PMID: 8078881), and members of the ansamycin family (formerly referred to as tyrosine kinase inhibitors) have been suggested as useful in effecting cancer therapy (Neckers L *et al.*, Invest New Drugs, 1999, 17(4): 361-73; PMID: 10759403; Schulte TW *et al.*, Cancer Chemother Pharmacol., 1998, 42(4): 273-9; PMID: 9744771).

One heat shock protein, Hsp90, has been implicated as involved in carcinoma of the breast, prostate, melanoma, leukaemias and lymphomas, colon and lung (Banerji U *et al.*, Curr Cancer Drug Targets, 2003 Oct; 3(5): 385-90; PMID: 14529390), as well as thyroid carcinomas. The role of Hsp90 is to ensure the correct folding of "client proteins" which are involved in a wide variety of cellular processes, for example signal transduction. Hsp90 client proteins include transcription factors such as mutant p53 and hypoxia-inducible factor 1α, and soluble kinases including v-Src, Akt, Raf-1, and Bcr-Abl. Hsp90 is constitutively expressed at 2- to 10-fold higher levels in tumour cells than in normal cells, suggesting that it may be important for the growth/survival of tumour cells (Schwartz, J., *et al,*. 2003, Semin. Hematol. 40:p87-96). Since the binding of client proteins to Hsp90 can regulate their conformation, stability and fate in the cell, Hsp90 can have a major impact on the pathways that regulate cellular outcome, including cell growth, division, differentiation, movement and death (Workman, P., Cancer Lett. 2004 Apr 8; 206(2):149-57; PMID: 15013520). The wide reaching

role for Hsp90 in cellular processes means the protein is currently viewed as a possible target for the development of therapeutic drugs. Hsp 90 inhibitors, by specifically interacting with a single molecular target, cause the destabilization and eventual degradation of Hsp90 client proteins.

A second aspect of the present invention relates to novel medicaments and preparations comprising effective anti-cancer agents together with an anti-Hsp90 antibody which together provide an enhanced efficacy in the treatment of leukaemia.

Leukaemia is a cancer that affects the bone marrow. In people with leukaemia, the bone marrow produces large numbers of abnormal white blood cells. The abnormal white blood cells crowd into the bone marrow, so the marrow can't make enough normal red blood cells, white blood cells and platelets.

Different types of leukaemia can be categorised by their speed of development (acute or chronic), and by the type of white blood cell affected, (myeloid or lymphoid cells). Myeloid white blood cells are the immune system's first line of defence against infection and are found mainly in the blood, where they engulf and kill foreign organisms. Lymphoid white blood cells are found in the lymph nodes and in the blood..

The four most common types of leukaemia include chronic lymphoid (lymphocytic) leukaemia (CLL), acute myeloid (myeloblastic) leukaemia (AML), acute lymphoid (lymphoblastic) leukaemia (ALL), and chronic myeloid leukaemia (CML).

CLL is also a cancer of the lymphocyte cells but develops more slowly than ALL. This disease is the most common type of leukaemia affecting adults, and is very rare in children.

AML is a cancer mainly affecting the myeloid cells known as granulocytes. It creates too many myeloblasts which can block blood vessels, and not enough mature myeloid cells. This disease occurs mainly in adults but can also affect children.

CML, (also called chronic granulocytic leukemia) is typically a slowly progressing cancer of the neutrophil cells, which is rare in children and commonly affects male adults more than females. CML is usually easily diagnosed because the leukaemic cells of more than 95% of patients have a distinctive cytogenetic abnormality, the Philadelphia chromosome (Ph1)

(Kurzrock, R. *et al.* 2003, Ann. Intern. Med. 138 (10): p819-30, PMID: 12755554; Goldman, J.M. and Melo, J.V., 2003, N. Engl. J. Med. 349 (15): p1451-64, PMID: 14534339). The Ph1 results from a reciprocal translocation between the long arms of chromosomes 9 and 22 and is demonstrable in all haematopoietic precursors (Deininger, M.W. *et al.* 2000, Blood 96 (10): p3343-56, PMID: 11071626). This translocation results in the transfer of the Abelson (abl) oncogene on chromosome 9, to an area of chromosome 22 termed the breakpoint cluster region (BCR) (Deininger, M.W. *et al.* 2000, Blood 96 (10): p3343-56, PMID: 11071626). This in turn results in a fused BCR/ABL gene which encodes an 8.5kb chimeric mRNA. The BCR/ABL gene is an oncogene which is sufficient to produce CML-like disease in mice. The transcript of the BCR/ABL oncogene is translated to yield a 210 kDa or 190 kDa protein. The Bcr-Abl protein is an abnormal tyrosine kinase protein that causes the disordered myelopoiesis found in CML. CML progresses through distinct clinical stages termed chronic phase, accelerated phase, and blast crisis. The BCR/ABL oncogene is expressed at all stages, but blast crisis is characterised by multiple additional genetic and molecular changes (Gorre, M.E., *et al.* 2002, Blood, 100(8): p3041-3044).

Ph1-negative CML is a rare disease that is characterized by the clinical characteristics of CML without cytogenetic or molecular (RT-PCR) evidence of the t(9;22)(q34;q11) translocation resulting in the Bcr-Abl fusion mRNA. Ph1-negative CML is a poorly defined entity that is less clearly distinguished from other myeloproliferative syndromes. Once thought to account for 5–10% of all clinical CML, with the routine accessibility of RT-PCR analysis for the Bcr-Abl transcript, that number is now well below 5%. Interestingly some patients with this entity may result from an alternative fusion to Abl. The TEL(ETV6)-ABL fusion, as a result of t(9;12), has been demonstrated in two cases of Ph- CML. Patients with Ph1-negative CML generally have a poorer response to treatment and shorter survival than Ph1-positive patients (Onida, F. *et al.* 2002: Cancer 95 (8): p1673-84, PMID: 12365015).

ALL is a cancer of immature lymphocyte cells, known as lymphoblasts. This disease is the most common type of leukaemia in young children, usually between the ages of 1 and 7 and is quite rare in adults. ALL causes many abnormal lymphocytes to be made, which crowd out the normal red blood cells and platelets. A 185 kDa Bcr-Abl protein has been directly implicated in the development in of ALL.

Two drugs, geldanamycin (GA), and 17-allylamino, 17-demethoxygeldanamycin (17-AAG) which act as Hsp90 inhibitors, have showed promising biological and clinical activity in clinical

trials. Indeed, the 210 kDa Bcr-Abl fusion protein ($p210^{Bcr-Abl}$) is dependent on its association with Hsp90 for its stability, and treatment of cells with GA or 17-AAG leads to rapid destruction of $p210^{Bcr-Abl}$.

An Hsp90 inhibitor such as 17AAG in combination with conventional cytotoxic agents or other novel agents, would also be therapeutically valuable in attacking multi step oncogenesis (Workman P., Cancer Lett. 2004 Apr 8; 206(2):149-57; PMID: 15013520). In cancer cells, which are characterised by genetic instability, it is possible that 17AAG, by blocking Hsp90 activity, releases a variety of mutations that together prove "synthetically lethal" to the tumour. Normal cells, which lack the tumour cells' genetic instability, are relatively unaffected (Garber, K., 2002, Journal of the National Cancer Institute, Vol. 94, No. 22, p1666-1668). A significant problem with 17AAG is that the drug is too toxic for prolonged therapy, and consequently there is a need for a non-toxic replacement (Banerji *et al.*, *supra*).

Imatinib mesylate (Gleevec (RTM)) is a small molecule tyrosine kinase inhibitor that has had a major impact on a neoplastic disease as a single agent. Originally designed as an inhibitor of the Bcr-Abl tyrosine kinase characteristic of malignancies carrying the pathogenic 9;22 translocation, Imatinib has proved to be moderately specific, and has made a major impact on the treatment of chronic myelogenous leukemia (CML) and Philadelphia chromosome positive (Ph1+) ALL (Krystal, GW, 2004, Leukemia Research 28S1:pS53-S59). One of the problems associated with Imatinib treatment of CML, is resistance to the drug as a result of mutations in the Bcr-Abl tyrosine kinase. Importantly, CML cells that have become resistant to Imatinib *in vivo* retain their Hsp90 dependence and thus remain sensitive to 17AAG.

Recent publications teach that tumour Hsp90 is present entirely in multi-chaperone complexes which facilitate malignant progression and that they are attractive targets for cancer therapeutics. In particular, Hsp 90 in multi-chaperone complexes derived from tumour cells is taught as having a 100-fold higher binding affinity for 17AAG than does Hsp90 from normal cells (i.e. Hsp90 in its latent uncomplexed state), indicating that in the multi-chaperone complex it may display epitopes (particularly quaternary epitopes) not displayed by the latent uncomplexed Hsp90. Mycograb (RTM) antibody can bind to Hsp 90 in its latent uncomplexed state, and also in multi-chaperone complexes, without any adverse effects on binding kinetics.

WO 01/76627 teaches compositions for treatment of fungal infections, the compositions comprising a combination of (I) a polyene or beta glucan synthase inhibitor antifungal agent; and (II) antibodies specific against fungal Hsp90, the compositions being effective against the fungus causing the infection despite its being resistant to the antifungal agent *per se*.

According to a first aspect of the present invention there is provided the use of:

(i) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(ii) at least one anti-cancer agent selected from the group consisting of: Doxorubicin, Daunorubicin, Epirubicin, Herceptin, Docetaxel, and Cisplatin,

in a method of manufacture of a medicament for the treatment of cancer.

Doxorubicin is an anthracycline antibiotic agent previously recognised as being an antitumour agent.

Epirubicin is a less toxic synthetic anthracyclin antibiotic, also previously recognised as being an antitumour agent.

Daunorubicin is an antineoplastic drug used in a number of therapeutic fields, including as an anti-cancer agent.

Herceptin (Trastuzumab) is a monoclonal antibody used for the treatment of HER2 protein overexpressing metastatic breast cancer.

Docetaxel is a recognised anti-cancer agent, and is a mitotic inhibitor.

Cisplatin is a recognised anti-cancer agent, and comprises a platinum complex.

As is detailed in the experimental results below ("Experiments A"), Doxorubicin and Daunorubicin are particularly preferred, and show particularly good synergistic effects with anti-Hsp90 antibody. Herceptin also shows good synergistic effects with anti-Hsp90 antibody. Synergy is also observed with Docetaxel and Cisplatin when combined with anti-Hsp90 antibody. The synergy between Daunorubicin and the antibody is particularly evident with oestrogen receptor positive cells, and so medicaments and therapies using the antibody and

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Daunorubicin may in particular be for (or administered to or for) cells having oestrogen receptors.

Experiments ("Experiments A") also show that other anti-cancer agents when used together with anti-Hsp90 antibody either show indifferent results (Paclitaxel) or antagonism (Imatinib). This confirms the surprising/unexpected nature of the synergy achieved with the above anti-cancer agents when combined with anti-Hsp90 antibody.

Also provided is a combined preparation comprising:

(i) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(ii) at least one anti-cancer agent selected from the group consisting of: Doxorubicin, Daunorubicin, Epirubicin, Herceptin, Docetaxel, and Cisplatin,

for simultaneous, separate or sequential use in the treatment of cancer.

Also provided is a method of treatment of cancer comprising administering a therapeutically effective quantity of:

(i) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(ii) at least one anti-cancer agent selected from the group consisting of: Doxorubicin, Daunorubicin, Epirubicin, Herceptin, Docetaxel, and Cisplatin,

to a patient in need of same.

As used herein, the term "treatment" is intended to have a broad meaning unless explicitly stated otherwise. Thus by "treatment" or "therapy" is meant any treatment which is designed to cure, alleviate, remove or lessen the symptoms of, or prevent or reduce the possibility of contracting disorders or malfunctions of the human or animal body. Thus by the term "treatment" is meant both treatment of disease conditions, as well as their prophylaxis.

The antibody or antigen binding fragment thereof may be specific for the epitope displayed by a peptide comprising the sequence of SEQ ID NO: 1.

As discussed above, although quaternary epitopes displayed by Hsp90 in multi-chaperone complexes have been suggested as appropriate targets for therapy, experiments (below) show that in fact a linear epitope is a useful and effective target for therapy.

Antibodies, their manufacture and uses are well known and disclosed in, for example, Harlow, E. and Lane, D., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999.

The antibodies may be generated using standard methods known in the art. Examples of antibodies include (but are not limited to) polyclonal, monoclonal, chimeric, single chain, Fab fragments, fragments produced by a Fab expression library, and antigen binding fragments of antibodies.

Antibodies may be produced in a range of hosts, for example goats, rabbits, rats, mice, humans, and others. They may be immunized by injection with fungal stress proteins, or any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase an immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminium hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Among adjuvants used in humans, BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum* are particularly useful.

Monoclonal antibodies to fungal proteins, or any fragment or oligopeptide thereof may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Koehler et al., 1975, Nature, 256: 495-497; Kosbor et al., 1983, Immunol. Today 4: 72; Cote et al., 1983, PNAS USA, 80: 2026-2030; Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss Inc., New York, pp. 77-96).

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison et al., 1984, PNAS USA, 81: 6851-6855; Neuberger et al., 1984, Nature, 312: 604-608; Takeda et al., 1985, Nature, 314: 452-454). Alternatively, techniques described for the production of single chain antibodies

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may be adapted, using methods known in the art, to produce fungal stress protein-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, D.R., 1991, PNAS USA, 88: 11120-11123).

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents (Orlandi et al., 1989, PNAS USA, 86: 3833-3837; Winter, G. et al., 1991, Nature, 349: 293-299).

Antigen binding fragments may also be generated, for example the F(ab')2 fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse et al., 1989, Science, 256: 1275-1281).

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between the fungal stress protein or any fragment or oligopeptide thereof, and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies specific to two non-interfering fungal stress protein epitopes may be used, but a competitive binding assay may also be employed (Maddox et al., 1983, J. Exp. Med., 158: 1211-1216).

For example, the antibody used in the composition or combined preparation may comprise the sequence of SEQ ID NO: 2.

The present inventor has found that cancers which may be usefully treated include fibrosarcomas and carcinomas selected from the group consisting: breast, prostate, melanoma, leukemia, lymphomas, leukemia, colon, testicular germ cell, pancreatic, ovarian, endometrial, thyroid, and lung.

According to a second aspect of the present invention there is provided the use of:

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(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90;

in a method of manufacture of a medicament for the treatment of leukaemia.

Also provided is the use of:

(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(II) at least one anti-cancer agent selected from the group consisting of: Imatinib, Paclitaxel, Docetaxel, Daunorubicin, Doxorubicin, and Hydroxyurea,

in a method of manufacture of a medicament for the treatment of leukaemia.

Imatinib, a derivative of 2-phenylaminopyrimidine, is a small molecule antagonist with activity against protein tyrosine kinases, and exhibits potent and specific inhibition of Bcr-Abl. Imatinib is indicated for the treatment of patients with CML in blast crisis, accelerated phase, or in chronic phase after failure of IFN- therapy.

Paclitaxel is chemotherapeutic agent that is given as a treatment for some types of cancer. It is most commonly used to treat ovarian, breast and non-small cell lung cancer.

Docetaxel is a recognised anti-cancer agent, and is a mitotic inhibitor.

Daunorubicin is an anti-neoplastic drug used in a number of therapeutic fields, including as an anti-cancer agent.

Doxorubicin is an anthracycline antibiotic agent previously recognised as being an anti-tumour agent.

Hydroxyurea is an anti-neoplastic, ribonucleotide reductase inhibitor.

As is detailed in the experimental results below ("Experiments B"), Doxetaxel and Paclitaxel are particularly preferred, and show particularly good synergistic effects with anti-Hsp90 antibody. Synergy is also observed with Imatinib, Doxorubicin, Daunorubicin, and Hydroxyurea when combined with anti-Hsp90 antibody. The anti-cancer agent Cisplatin, when used together with anti-Hsp90 antibody showed indifferent results. This confirms the surprising/unexpected nature of the synergy achieved with the above anti-cancer agents when combined with anti-Hsp90 antibody.

Also provided is a combined preparation comprising:

(i) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(ii) at least one anti-cancer agent selected from the group consisting of: Imatinib, Paclitaxel, Docetaxel, Daunorubicin, Doxorubicin, and Hydroxyurea,

for simultaneous, separate or sequential use in the treatment of leukaemia.

Examples of combined preparations include pharmaceutical packs containing the antibody of (i) and at least one anti-cancer agent of (ii) in separate volumes (i.e. not mixed together in a single preparation).

Also provided is a method of treatment of leukaemia comprising administering a therapeutically effective quantity of:

(i) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(ii) at least one anti-cancer agent selected from the group consisting of: Imatinib, Paclitaxel, Docetaxel, Daunorubicin, Doxorubicin, and Hydroxyurea,

to a patient in need of same.

The leukaemia may be chronic myeloid leukaemia or acute lymphoid leukaemia, and the at least one anti-cancer agent may Imatinib.

The antibody or antigen binding fragment thereof may be specific for the epitope displayed by a peptide comprising the sequence of SEQ ID NO: 1.

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For example, the antibody used in the composition or combined preparation may comprise the sequence of SEQ ID NO: 2.

The anti-cancer agent may be Imatinib.

The present inventor has found that leukaemias which may be usefully treated include leukaemias selected from the group consisting of: acute myeloblastic leukaemia, acute lymphoblastic leukaemia, chronic myeloid leukaemia, and chronic lymphocytic leukaemia.

The leukaemia may be chronic myeloid leukaemia or acute lymphoid leukaemia.

The chronic myeloid leukaemia may be Ph1-positive or Ph1-negative, I.e is characterized by leukaemic cells which contain the Philadelphia chromosome (Ph1-positive), or lack the Philadelphia chromosome (Ph1-negative).

The present inventor has found that chronic myeloid leukaemias which may be usefully treated with Imatinib may be either Ph1-positive or Ph1-negative.

The leukaemia may be chronic myeloid leukaemia which is Ph1-positive, and the anti-cancer agent may be Imatinib. In particular, the present inventor has found that treatment of CML which is Ph1-positive can be effected by a combination of Imatinib and an antibody comprising the sequence of SEQ ID NO: 2. Without wishing to be bound by any theory, it is possible that Hsp90 is sequestered by the antibody comprising the sequence of SEQ ID NO: 2, which in turn means that the abnormal Bcr-Abl tyrosine kinase (which causes the disordered myelopoiesis found in CML) is e.g. incorrectly folded, targeted for protein degradation, and/or prevented from exerting it's effects on myelopoietic pathways.

This treatment is further effective on Imatinib resistant CML Ph1-positive cells. Without wishing to be bound by any theory, the resistance to Imatinib is likely to be due to collected mutations in the abnormal Bcr-Abl tyrosine kinase which could e.g. prevent the drug from binding to the protein and/or interfere with the mode of action of the drug. In Imatinib resistant cells, it is possible that the sequestration of Hsp90 by the antibody comprising the sequence of SEQ ID NO: 2, causes the mutated abnormal tyrosine kinase to be e.g. incorrectly folded, targeted for protein degradation, and/or prevented from exerting it's effects on myelopoietic pathways. The sequestration of Hsp90, which normally serves to "buffer" the genetic

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mutations associated with cancerous cells by binding to abnormal proteins and blocking their expression, may also cause a variety of mutations to be released which together prove synthetically lethal to the tumour cell. Normal cells, which lack the tumour cells' genetic instability, are relatively unaffected.

Additionally, and particularly surprisingly, the present inventor has found that treatment of CML which is Ph1-negative can be effected by a combination of Imatinib and an antibody comprising the sequence of SEQ ID NO: 2.

The leukaemia may be chronic myeloid leukaemia which is Ph1-negative, and the anti-cancer agent may be Imatinib.

This finding is surprising because Ph1-negative CML cells lack the abnormal tyrosine kinase protein associated with Ph1-positive cells. However, and without wishing to be bound by any theory, it is possible there are low or basal levels of this kinase (whether abnormal or otherwise) in Ph1-negative cells, and as described above, the sequestration of Hsp90 is by the antibody comprising the sequence of SEQ ID NO: 2, means that the tyrosine kinase protein is e.g incorrectly folded, or targeted for protein degradation, or in some way prevented from exerting its effects on myelopoietic pathways. It may also be the case that by sequestering Hsp90, a variety of mutations are released by the tumour cell which together prove synthetically lethal.

The surprising effect of Imatinib and the anti-Hsp90 antibody in Ph1-negative cells may be due to the presence of the TEL(ETV6)-ABL fusion, which has been demonstrated in two cases of Ph1-negative CML (Krystal, GW, 2004, Leukemia Research 28S1:pS53-S59), and which is sensitive to Imatinib.

The leukaemia may be characterised by cells which are Imatinib resistant.

The composition or preparation of the present invention may additionally comprise a known Hsp 90 inhibitor, for example GA, or 17-AAG.

A third aspect of the present invention (Experiments C) relates to novel medicaments and preparations comprising effective anti-cancer agents together with anti-Hsp90 antibody which together provide an enhanced efficacy in the treatment of colorectal cancer or

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adenocarcinomas.

Colorectal cancer is a malignant tumour of the colon or rectum. Colorectal cancer is a leading cause of cancer morbidity and mortality. It is the third most common cancer in men and the second most common cancer in women in the UK. Ninety five percent of colorectal cancers are adenocarcinomas, which are cancers of the glandular cell that line the inside of the colon and rectum.

Standard treatment of colorectal cancer is usually a combination of 5-fluorouracil and leucovorin (folinic acid).

5-fluorouracil (5-FU) is used to treat a number of solid tumours, including gastro-intestinal cancers and breast cancer. It is commonly used with folinic acid in advanced colorectal cancer. 5-FU is converted to FdUMP in the cell, which forms a complex with Thymidylate synthase (TS) inhibiting DNA, protein and RNA synthesis.

Folinic acid (Leucovorin) is a vitamin which is given in combination with 5-FU. Folinic acid increases the response rate to 5-fluorouracil, with a significant improvement in disease free and overall survival. Folinic acid increases the intracellular folate and stabilises the FdUMP/TS complex.

Other agents found to have an effect include irinotecan and oxaliplatin, which is licensed for first-line use in patients with advanced colorectal cancer, in combination with 5-fluorouracil and folinic acid. Irinotecan or raltitrexed are licensed for use as a second-line monotherapy when fluorouracil-based therapy has failed or is inappropriate.

Oxaliplatin is a recognised anti-cancer agent and contains a novel diaminocyclohexane platinum compound which forms cross-links in DNA and so inhibits DNA replication.

'FOLFOX' is the commonly used combination chemotherapy of 5-fluorouracil, folinic acid and Oxaliplatin.

Irinotecan (CPT-11, Campto) inhibits topoisomerase I, a DNA-unwinding enzyme essential for cell division, which results in replication arrest with breaks in single-strand DNA. In the UK, Irinotecan is licensed for use in chemotherapy-naïve patients with advanced colorectal cancer

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in combination with 5FU/FA and as a single agent for second-line chemotherapy in patients who have failed an established 5FU-based regimen.

Raltitrexed (ZD 1694, Tomudex) inhibits the enzyme thymidylate synthetase, which is involved in DNA synthesis. This is the same enzyme that is targeted by 5FU. Raltitrexed is licensed in the UK for the palliative treatment of advanced colorectal cancer where 5FU/FA-based regimens are either not tolerated or inappropriate.

Tebbutt *et al.*, 2002, European Journal of Cancer, 38: 1000-1015; Cutsem *et al.*, 2002, Best Practice and Research Clinical Gastroenterology, 16: 319-330; Beretta *et al.*, 2004, Surgical Oncology, 13: 63-73; NICE guidelines for Irinotecan, Oxaliplatin and raltitrexed for advanced colorectal cancer, 2002.

According to this third aspect of the invention, there is provided the use of:

(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(II) at least one anti-cancer agent selected from the group consisting of: 5-fluorouracil, oxaliplatin, irinotecan and raltitrexed,

in a method of manufacture of a medicament for the treatment of cancer.

Alternatively, there is provided a combined preparation comprising:

(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(II) at least one anti-cancer agent selected from the group consisting of:, 5-fluorouracil, oxaliplatin, irinotecan and raltitrexed,

for simultaneous, separate or sequential use in the treatment of cancer.

According to yet a further aspect of this invention, there is provided a method of treatment of cancer comprising administering a therapeutically effective quantity of:

(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(II) at least one anti-cancer agent selected from the group consisting of: 5-fluorouracil, oxaliplatin, irinotecan and raltitrexed,

to a patient in need of same.

Preferably, the cancer is colorectal cancer or adenocarcinoma.

Most preferably, the anti-cancer agent 5-fluorouracil further comprises or is administered with folonic acid (leucovorin).

Additionally or alternatively, 5-fluorouracil, folinic acid and oxaliplatin are administered together.

The composition or preparation according to any aspect of this invention may additionally comprise a pharmaceutically acceptable carrier, diluent or excipient. Similarly, any method of manufacture of the present invention or use in same may also comprise the use of a pharmaceutically acceptable carrier, diluent or excipient. Examples of pharmaceutically acceptable carriers, diluents and excipients are well known in the art, for example see: Remington's Pharmaceutical Sciences and US Pharmacopoeia, (1984, Mack Publishing Company, Easton, PA, USA).

The medicaments or combined preparation may, for example, be administered orally although this does not mean that other administration routes are to be excluded.

The antibody or antigen binding fragment thereof according to the present invention may be labelled with a detectable label or may be conjugated with an effector molecule, for example a drug e.g. an anti-cancer agent such as Doxorubicin, Daunorubicin, Docetaxel, or Cisplatin, or 5-fluorouracil, oxaliplatin, irinotecan and raltitrexed or a pharmaceutical agent useful in treating leukaemia e.g. Imatinib, Paclitaxel, Docetaxel, Daunorubicin, Doxorubicin, and Hydroxyurea, or a toxin, such as ricin, or an enzyme, using conventional procedures, and the invention extends to such labelled antibodies or antibody conjugates.

If desired, mixtures of antibodies may be used for diagnosis or treatment, for example mixtures of two or more antibodies recognising different epitopes of a stress protein according to the invention, and/or mixtures of antibodies of a different class, e.g. mixtures of IgG and IgM antibodies recognising the same or different epitope(s) of the invention.

As discussed above, although quaternary epitopes displayed by Hsp90 in multi-chaperone complexes have been suggested as appropriate targets for therapy, experiments (below) show that in fact a linear epitope is a useful and effective target for therapy.

The contents of each of the references discussed herein, including the references cited therein, are herein incorporated by reference in their entirety.

Where "PMID:" reference numbers are given for publications, these are the PubMed identification numbers allocated to them by the US National Library of Medicine, from which full bibliographic information and abstract for each publication is available at www.ncbi.nlm.nih.gov. This can also provide direct access to electronic copies of the complete publications, particularly in the case of e.g. PNAS, JBC and MBC publications.

The present invention will be further apparent from the following description, which shows, by way of example only, specific embodiments of the composition and experimentation therewith.

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EXPERIMENTS

A first set of experiments ("Experiments A") described below detail the investigation of the anti-cancer effect of an anti-Hsp90 antibody having the sequence of SEQ ID NO: 2 and specific for an epitope displayed by a peptide having the sequence of SEQ ID NO: 1, used on its own or in combination with the anti-cancer agents Doxorubicin, Daunorubicin, Docetaxel, Herceptin, Imatinib, Cisplatin, and Paclitaxel.

A second set of experiments ("Experiments B") described below detail the investigation of the effect of an anti-Hsp90 antibody having the sequence of SEQ ID NO: 2 and specific for an epitope displayed by a peptide having the sequence of SEQ ID NO: 1, used on its own or in combination with the anti-cancer agents Imatinib, Paclitaxel, Docetaxel, Daunorubicin, Doxorubicin, Paclitaxel, Cisplatin, and Hydroxyurea, on the human Caucasian chronic myelogenous leukaemia cell line K562, and human myelogenous leukaemia cell line KU-812.

A third set of experiments, ("Experiments C") describe below in detail the investigation of the effect of an anti-Hsp90 antibody having the sequence of SEQ ID NO: 2 and specific for epitope displayed by a peptide having the sequence of SEQ ID NO: 1, used on its own or in combination with the anti-cancer agent's 5-Fluorouracil (5-FU) and Folinic acid (Leucovorin, LV) and/or Oxaliplatin on the human colon adenocarcinoma cell line HT29.

GENERAL MATERIALS AND METHODS

Unless stated otherwise, all procedures were performed using standard protocols and following manufacturer's instructions where applicable. Standard protocols for various techniques including PCR, molecular cloning, manipulation and sequencing, the manufacture of antibodies, epitope mapping and mimotope design, cell culturing and phage display, are described in texts such as McPherson, MJ *et al.* (1991, PCR: A practical approach, Oxford University Press, Oxford), Sambrook, J. and Russell, D., "Molecular Cloning: A Laboratory Manual", Third Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, New York, 2001, Huynh and Davies (1985, "DNA Cloning Vol I - A Practical Approach", IRL Press, Oxford, Ed. DM Glover), Sanger, F. *et al.* (1977, PNAS USA 74(12): 5463-5467), Harlow, E. and Lane, D. ("Using Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, New York, 1998), Jung, G. and Beck-Sickinger, AG (1992, Angew. Chem. Int. Ed. Eng., 31: 367-486), Harris, M.A. and Rae, I.F. ("General Techniques of Cell Culture", 1997,

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Cambridge University Press, ISBN 0521 573845), "Phage Display of Peptides and Proteins: A Laboratory Manual" (Eds. Kay, BK, Winter, J., and McCafferty, J., Academic Press Inc., 1996, ISBN 0-12-402380-0).

Reagents and equipment useful in, amongst others, the methods detailed herein are available from the likes of Amersham (www.amersham.co.uk), Boehringer Mannheim (www.boehringer-ingelheim.com), Clontech (www.clontech.com), Genosys (www.genosys.com), Millipore (www.millipore.com), Novagen (www.novagen.com), Perkin Elmer (www.perkinelmer.com), Pharmacia (www.pharmacia.com), Promega (www.promega.com), Qiagen (www.qiagen.com), Sigma (www.sigma-aldrich.com) and Stratagene (www.stratagene.com).

Antibody

The antibody used in Experiments A and B below is that disclosed in WO 01/76627, and is herein referred to as Mycograb (RTM), having the sequence of SEQ ID NO: 2 and being specific for an epitope displayed by the peptide having the sequence of SEQ ID NO: 1. The basic antibody solution was a 4 mg/ml stock solution in water. Further dilutions were carried out in RPMI complete medium.

Briefly, the DNA sequence of a former antibody specific for the *Candida albicans* Hsp90 epitope disclosed in GB 2240979 and EP 0406029 was genetically modified by codon optimisation for expression in *Escherichia coli* (Operon Technologies Inc., Alameda, CA, USA) and inserted into an *E. coli* expression vector. The amino acid sequence of the anti-Hsp90 antibody comprises the sequence of SEQ ID NO: 2 (includes the heavy, light and spacer domains). The antibody recognises the epitope comprising the sequence of SEQ ID NO: 1.

The anti-Hsp90 antibody was expressed in an *Escherichia coli* host and then purified by affinity chromatography and an imidazole exchange column up to 95 % purity. Standard molecular biology protocols were employed (see, for example, Harlow & Lane, *supra*; Sambrook, J. *et al.*, 1989, Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Sambrook, J. & Russell, D., 2001, Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor).

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Drugs

Cisplatin was obtained from Bristol-Myers Squibb, Mayne, supplied as 1 mg/ml.

Docetaxel was obtained from Sigma. 5 mg was diluted initially to 16 mg/ml with Dimethyl sulfoxide (DMSO).

Doxorubicin was obtained from Pharmacia; 5 ml supplied as Doxorubicin hydrochloride 2 mg/ml.

Imatinib (Glivec (RTM)), obtained from Novartis, was supplied as 100 mg capsule. Imatinib was initially diluted in water to produce a 10 mg/ml stock solution.

Paclitaxel was obtained from Sigma, reconstituted in 250 µl methanol made up to 2.5 ml with water to give 2 mg/ml.

Daunorubicin was obtained from Sigma, 5 mg was diluted in 2.5 ml water to give 2 mg/ml.

Herceptin (RTM) (Trastuzumab) was obtained from Roche and reconstituted in 7.2 ml of water to give 21 mg/ml.

Hydroxyurea was obtained from Sigma, with 1g diluted in 4 ml water to give 25 mg/ml.

5-Fluorouracil (5-FU) was obtained from Sigma, 96mg was reconstituted in 1ml DMSO diluted 1/10 in complete RPMI media to give 9.6mg/ml.

Folinic acid (LV) was obtained from Sigma; 100mg was reconstituted in 25ml of water to give a 4mg/ml stock solution.

Oxaliplatin was obtained from Sigma; 12.5mg was reconstituted in 2.5ml of water to give 5mg/ml.

Each of the above drugs was further diluted in RPMI complete medium.

Cell concentration and viability determination

Cells were counted and percentage viability determined using a standard haemocytometer following staining with an equal volume of 0.4% Trypan Blue solution (Sigma).

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Cell Viability Assay

Cell viabilities were assessed after each experiment using the Cell Titer Blue Assay (Promega). Media was removed from the cells and 100 µl of fresh complete medium added followed by 20 µl of Cell Titer Blue Reagent. This was incubated at 37 °C, 5% CO_2 for 4 hours and absorbance read at 570 nm using 600 nm as a reference. This assay uses the indicator dye resazurin (blue) to measure the metabolic capacity of the cells. Viable cells reduce resazurin to resorufin (pink).

Data Interpretation

Cell growth was evaluated as described above. The IC_{50} (the dose of drug needed to cause cytotoxicity in 50% of the cells) concentrations were determined singly for each drug over 48 hour incubation periods.

Median effect analysis, a measure of synergism, additive effects, or antagonism based upon the Hill equation, was determined by the method of Chou and Talalay using the Calcusyn product (BioSoft, Cambridge, UK - www.biosoft.com). The CI (combination index) which reflects synergy when less than 1, additive effects when equal to 1, and antagonism when greater than 1 was calculated for varying levels of drug effect. Ten fixed drug ratios above and below the IC_{50} (the concentration of drug required to exert a 50% cytotoxic effect) with a range of 0.0156N-8N where N is a value near the IC_{50} of an individual drug were explored by incubating the drug combinations with cells for 48 hours and then determining the degree of cytotoxicity. Fa50 is defined at that point where 50% of the cells are affected. CI values are shown for Fa50.

EXPERIMENTS A

The results show that the antibody gave evidence of antagonism with Imatinib and indifference with Paclitaxel. There was some synergy with Cisplatin and with Docetaxel, but the latter is probable at concentrations which cannot be achieved clinically. Doxorubicin demonstrated synergy at clinically achievable drug levels with both cell lines and independently of whether there was an oestrogen receptor. The results achieved with Doxorubicin rate as a highly significant synergy. The results of Daunorubicin were equally impressive with the cell line with an oestrogen receptor but less with the oestrogen receptor negative cell line with synergy restricted to 6 and 12.5 mg/l. The results for Herceptin showed

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no synergy against an oestrogen receptor positive cell line, but synergy was observed with an oestrogen receptor negative cell line.

Materials and Methods

Cell line and culture information

Human Caucasian breast adenocarcinoma cell line MCF7, expressing both wild type and variant oestrogen receptors as well a progesterone receptor, was obtained from ECACC (ECACC number - 86012803).

Other cell lines used are as follows:

HS578T - ECACC number 86082104, Human breast carcinoma, Epithelial. Tumorigenic in immunosuppressed mice and form colonies in semisolid medium. Oestrogen receptor negative.

SK-BR-3 - (ATCC) Human breast adenocarcinoma. Oestrogen receptor positive. Over expresses HER2/C-erb-2 gene.

UACC-812 - (ATCC) Ductal Carcinoma, prior to surgery, patient had extensive chemotherapy. Oestrogen receptor negative, progesterone receptor negative, P-glycoprotein negative. Amplification of HER-2/ neu oncogene sequence

HCT116 - ATCC Colorectal carcinoma. Positive for TGF Beta 1 and beta 2 expression

Cells were split using 0.25% trypsin/EDTA (Sigma) and maintained in RPMI medium without phenol red, containing 10% Foetal Bovine Serum, 1% Non Essential Amino Acids, 2 mM Glutamine, 100 U/ml Penicillin, 0.1 mg/ml Streptomycin (Sigma) at 37 °C, 5% CO_2.

Experiments A

Effect of Mycograb on MCF7 cells

The cell lines were split and cells counted. Cells were added to 12- or 96- well flat-bottomed tissue culture plates. In the case of the 12- well plates, 1 ml of $4x10^4$ cells/ml were added plus 1 ml of medium. In the case of the 96- well plate, 100 µl of $4x10^4$ cells/ml were added followed by a further 100 µl of complete medium was added to the plate. The plates were incubated overnight at 37 °C, 5% CO_2. The next day, the cells were observed under phase contrast microscopy to ensure they had adhered to the plates and the supernatant medium removed by aspiration. Fresh medium containing twofold increasing concentrations of

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Mycograb (1.5 - 200 µg/ml) or formulation buffer or medium alone was then added to the wells. The plate was returned to the incubator for 48 hours following which the cell titre blue assay was carried out or viable counts were carried out using a haemocytometer.

Effect of anti-cancer agents Doxorubicin, Daunorubicin, Herceptin, Docetaxel, Imatinib, Paclitaxel and Cisplatin on MCF7 cells

The cell lines were split and cells counted. 100 µl of $4x10^4$ cells/ml were added to 96 well flat bottomed tissue culture plates a further 100 µl of complete medium was added to the plate. The plates were then incubated overnight at 37°C, 5% CO_2. The next day, the cells were observed under phase contrast microscopy to ensure they had adhered to the plates and the supernatant medium removed by aspiration. Fresh medium containing increasing concentrations of study drug (Doxorubicin 0.55-600 µg/ml, Daunorubicin 0.45-1000 µg/ml, Herceptin 0.2-200 µg/ml, Docetaxel 0.75-800 µg/ml, Imatinib 4.5-5000 µg/ml, Cisplatin 0.04-50 µg/ml, Paclitaxel 1.8-1000 µg/ml) or medium alone was added to the wells. The plates were returned to the incubator for 48 hours following which cell titre blue assays were carried out.

Effect of Mycograb in combination with anti-cancer agents Doxorubicin, Daunorubicin, Herceptin, Docetaxel, Imatinib, Paclitaxel and Cisplatin on MCF7 cells

The cell lines were split and cells counted. 100 µl of $4x10^4$ cells/ml were added to 96 well flat bottomed tissue culture plates a further 100 µl of complete medium was added to the plate. The plates were then incubated overnight at 37 °C, 5% CO_2. The next day, the cells were observed under phase contrast microscopy to ensure they had adhered to the plates and the supernatant medium removed by aspiration. 100 µl of fresh medium was added to the plates and a checkerboard of Mycograb versus other drug was set out as outlined in Table 1 below (using Doxorubicin as an example) to give a total volume of 200 µl per well.

The plates were returned to the incubator for 48 hours following which cell titer blue assays were carried out.

Experiments were also performed with HS578T cells using the above methodologies, and results are given below.

Results of Experiments A

MCF7 cell line

Cisplatin

The IC_{50} was 6 mg/l and there was no effect on adding Mycograb with the exception of synergy at higher concentrations - see Table 2.

Imatinib

The IC_{50} was 37.5 mg/l and there was evidence of antagonism with Cis in the range of 3.3-10 with Mycograb at doses of Imatinib below 37.5 mg/l. Above this dose the Imatinib killed the cell line.

Docetaxel

The IC50 was 225 mg/l and there was evidence of some synergy with Mycograb at high doses of Docetaxel - see Table 3.

Paclitaxel

The IC_{50} was 225 mg/l and there was indifference with low concentrations of the drug and mild synergy at high levels such as 500 mg/l of Paclitaxel. These levels are outside those that are clinically relevant.

Doxorubicin

The IC_{50} was 1.75 mg/l. There was clear synergy with Mycograb over a range of drug concentrations - see Table 4.

Daunorubicin

The IC_{50} was 1 mg/l. There was evidence of synergy with Mycograb over a range of drug concentrations - see Table 5.

Herceptin

There was no detectable activity due to Herceptin and no evidence of synergy.

HS578T cell line

This cell line was insensitive to Mycograb in increasing concentrations up to 400 mg/l. This was not surprising in that these tumours are not steroid sensitive and thus not intrinsically

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likely to respond to an Hsp90 inhibitor such as Mycograb. However in combination with the anthracycline Doxorubicin, as well as Daunorubicin, and Herceptin there was unexpected synergy.

Doxorubicin

The IC50 was 1 mg/l. There was evidence of synergy with Mycograb over a range of drug concentrations - see Table 6.

Daunorubicin

The IC_{50} was 1 mg/l. There was some evidence of synergy but mostly indifference with Mycograb - see Table 7.

Herceptin

With HS578T, mono-herceptin failed to kill 50% of the cells in concentrations of up to 200 mg/l, but in the presence of Mycograb synergy was observed - see Table 8.

Docetaxel

This gave an IC_{50} of 50 mg/l for the cell line HS578T and showed no evidence of synergy with Mycograb.

Cisplatin

Cisplatin had an IC_{50} of 12.5 mg/l for the cell line HS578T and showed no evidence of synergy with Mycograb

Conclusion

There was evidence of antagonism with Imatinib and indifference with Paclitaxel. There was some synergy with Cisplatin and with Docetaxel, but the latter is probable at concentrations which cannot be achieved clinically. Doxorubicin demonstrated synergy at clinically achievable drug levels with both cell lines and independently of whether there was an oestrogen receptor. The results achieved with Doxorubicin rate as a highly significant synergy. The results of Daunorubicin were equally impressive with the cell line with an oestrogen receptor but less with the oestrogen receptor negative cell line with synergy restricted to 6 and 12.5 mg/l.

This above surprising synergistic effect is observed between the antibody and certain anti-cancer drugs, but not with other anti-cancer agents such as Imatinib.

EXPERIMENTS B

A second set of experiments (described below) detail the investigation of the effect of an anti-Hsp90 antibody having the sequence of SEQ ID NO: 2 and specific for an epitope displayed by a peptide having the sequence of SEQ ID NO: 1, used on its own or in combination with the anti-cancer agents Imatinib, Paclitaxel, Docetaxel, Daunorubicin, Doxorubicin, Paclitaxel, Cisplatin, and Hydroxyurea, on the human Caucasian chronic myelogenous leukaemia cell line K562, and human myelogenous leukaemia cell line KU-812.

The results show that the antibody gave evidence of synergy with Imatinib, Paclitaxel, Docetaxel, Daunorubicin. The antibody gave evidence of some synergy with Doxorubicin and Hydroxyurea. The results show that the antibody gave evidence of indifference with Cisplatin.

The results achieved with Docetaxel and Paclitaxel rate as a highly significant synergy.

Material and Methods

Cell line and culture information

Human myelogenous leukaemia cell line KU-812 was obtained from ECACC (ECAAC number 90071807). A Philadelphia chromosome (Ph1) has been detected in this cell line. The cells are morphologically characteristic of basophils.

Human Caucasian chronic myelogenous leukaemia cell line K562, was obtained from ECACC (ECACC number 89121407). K562 was established from pleural effusion of 53 year old female with chronic myelogenous leukaemia in terminal blast crisis. Karyological studies on various K-562 sublines have been classified into three groups (A, B, C) (Dimery, I. W. *et al.*, 1983, Exp. Hematol.;11(7):p601-10). The line used in these experiments was the K562B. Experiments have demonstrated that these lines are generally similar in terms of: morphology, growth kinetics in liquid suspension culture, cloning efficiency in soft agar culture, binding of anti-K562 monoclonal antibodies, and cell surface proteins. K562B has been compared to K562A and K562 C, with respect to growth kinetics, cell surface protein markers, surface antigens, cytogenetics and hemoglobin production. Differences were observed between the cell lines, the most important difference being that whereas more than

90% of K562A or C cells appeared to be Ph1-positive, less than 15% of K562B cells contained a Ph1 (Dimery, IW, *et al.*, 1983, Exp. Hematol.;11(7):p601-10). Although cytogenetic tests do not reveal the presence of a true Ph1 chromosome K562 appears to contain part of a Ph1 chromosome, which is at least fourfold amplified. This part of a Ph1 chromosome encodes a chimeric *bcr/c-abl* transcript, which when translated yields a *bcr/c-abl* fusion protein (Grosveld, G., et al., 1986, Mol. Cell. Biol. 6, No. 2: p607-616). The *bcr/c-abl* fusion protein possesses activated tyrosine kinase activity which is responsible for the pathogenesis of CML.

Cells were maintained between 2 x 10^5 and 9 x 10^5 cell/ml in RPMI medium 1640 without phenol red, containing 10% Foetal Bovine Serum, 2 mM Glutamine, 100 U/ml Penicillin, 0.1 mg/ml Streptomycin (Sigma) at 37 °C, 5% CO_2.

Experiments

Effect of Mycograb on K562 Cells

The cell lines were counted. Cells were added to 96 well flat-bottomed tissue culture plates using aliquots of 100 µl containing 4 x 10^5 cells/ml. Fresh medium containing either two-fold increasing concentrations of Mycograb (RTM) (1.5 - 200 µg/ml), or medium alone was then added to the wells. The plate was returned to the incubator for 48 hours following which the cell titre blue assay was carried out, or viable counts were determined using a haemocytometer.

Effect of anti-cancer agents Doxorubicin, Daunorubicin, Docetaxel, Paclitaxel, Imatinib, Cisplatin and Hydroxyurea on K562 cells

The cell lines were counted. 100 µl of 2 x 10^5 or 4 x 10^5 cells/ml were added to 96 well flat bottomed tissue culture plates. The plates were then incubated overnight at 37 °C, 5% CO_2. Fresh medium containing increasing concentrations of study drug (Doxorubicin 0.55-600 µg/ml, Daunorubicin 0.07-100 µg/ml, Docetaxel 0.75-800 µg/ml, Paclitaxel 0.5-500 µg/ml, Imatinib 4.5-5000 µg/ml, Cisplatin 0.04-50 µg/ml) or medium alone was added to the wells. The plates were returned to the incubator for 48 hours following which cell titre blue assays were carried out.

Effect of Mycograb in combination with anti-cancer agents Doxorubicin, Daunorubicin, Docetaxel, Paclitaxel, Imatinib, Cisplatin and Hydroxyurea on K562 cells

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The cell lines were counted. 100 µl of 2 x 10^5, or 4 x 10^5 cells/ml were added to 96 well flat bottomed tissue culture plates. The plates were then incubated overnight at 37 °C, 5% CO_2. 100 µl of fresh medium was added to the plates and a checkerboard of Mycograb versus other drug was set out as outlined in Table 9 below (using Doxorubicin as an example) to give a total volume of 200 µl per well.

Experiments were also performed with KU-812 cells using the above methodologies, and results given below.

Results

Effect of Mycograb (RTM) on K562 Cells

With cell line K562 Mycograb on its own at 12.5µg/ml demonstrated a 40% reduction in cell viability.

Effect of Mycograb (RTM) and anti-cancer agents on K562 Cells

Imatinib

The IC_{50} was 16 µg/ml. There was some evidence of synergy between Imatinib and Mycograb (RTM) at a range of drug concentrations (see Table 10).

Doxorubicin

The IC_{50} was 1 µg/ml. There was some evidence of synergy between Doxorubicin and Mycograb (RTM) at some drug concentrations but mostly indifference with Mycograb (RTM).

Daunorubicin

The IC_{50} was 0.75 µg/ml. There was some evidence of synergy between Daunorubicin and Mycograb (RTM) at low of drug concentrations (see Table 11).

Docetaxel

The IC_{50} was 70 µg/ml. There was clear evidence of synergy between Docetaxel and Mycograb (RTM) at a range of drug concentrations (see Table 12).

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Paclitaxel

The IC_{50} was 32 µg/ml. There was clear evidence of synergy between Paclitaxel and Mycograb (RTM) at a range of drug concentrations (see Table 13).

Cisplatin

The IC_{50} was 12.5 µg/ml. There was no evidence of synergy between Cisplatin and Mycograb (RTM).

Hydroxyurea

The IC_{50} was never reached with Hydroxyurea as the sole agent. However, there was some evidence of synergy between Hydroxyurea and Mycograb (RTM) at low of drug concentrations (see Table 14).

KU-812 Cell line

Effect of Mycograb (RTM) on KU-812 Cells

With cell line KU-812 Mycograb on its own at 50µg/ml demonstrated a 40% reduction in cell viability.

Effect of Mycograb (RTM) and anti-cancer agents on KU-812 Cells

Imatinib

The IC_{50} was 0.12 µg/ml. There was some evidence of synergy between Imatinib and Mycograb (RTM) at low of drug concentrations (see Table 15).

Summary

Using a K562 cell line, there was evidence of synergy with Imatinib, Paclitaxel, and Docetaxel. There was evidence of some synergy with Daunorubicin, Doxorubicin and Hydroxyurea. Indifference was seen with Cisplatin.

Using a KU-812 cell line, there was evidence of some synergy with Imatinib.

Conclusions

The data presented here clearly demonstrates that Mycograb (RTM) antibody on it's own can decrease the viability of both Ph1-positive and Ph1-negative CML cell lines. Furthermore, there is a surprising synergism between anti-cancers agents, including Imatinib and the anti-Hsp90 antibody, in Ph1-positive CML cell lines. The data also demonstrate that there is

synergism between Paclitaxel, Docetaxel, Daunorubicin, Doxorubicin, Hydroxyurea, and the anti-Hsp90 antibody, in Ph1-positive leukaemia cell lines. These results allows for the use of compositions comprising anti-cancer agents such as Imatinib, together with the anti-Hsp90 antibody (Mycograb, RTM) for the treatment of CML. The synergism exhibited by the combination of anti-cancer agent and Mycograb (RTM) antibody potentially allows for either lower treatment dosages, which would be hugely significant given the problematic toxicity of many of the anti-cancer agents, and in particular Imatinib, or more effective and longer treatments at the same dosages, thereby reducing unwanted side-effects.

Clinical implications of the present invention include: (i) the production of a synergistic combination of anti-cancer agents e.g. Imatinib, and anti-Hsp90 antibody in the treatment of CML should become the treatment of choice. This would possibly lead to a reduction in mortality for CML; (ii) Imatinib is toxic, and the synergy provided by the present invention means that a lower dose of Imatinib could be used while maintaining efficacy and concomitantly reducing toxicity; and (iii) the toxicity sparing effect of the anti-hsp90 antibody would allow the clinical efficacy of higher doses of Imatinib to be explored and further contribute to an improved clinical outcome.

EXPERIMENTS C

A third set of experiments (described below) detail the investigation of the effect of an anti-Hsp90 antibody having the sequence SEQ ID NO: 2 and specific for the epitope displayed by a peptide having the sequence SEQ ID NO: 1, used on its own or in combination with the anti-cancer agents 5-FU and Folinic acid and/or Oxaliplatin on the human colon adenocarcinoma cell line HT29.

The results show that the antibody gave evidence of synergy with 5-FU and Folinic acid and with Oxaliplatin. There was also evidence of synergy with the four drug combination of Mycograb/anti-Hsp90 antibody with 5-FU, Folinic acid and Oxaliplatin. Concentrations of greater then 75µg/ml of 5-FU and 10.5µg/ml of Oxaliplatin were found to be particularly useful.

Material and Methods

Cell line and culture information

Human Caucasian colon adenocarcinoma grade II cell line HT29, was obtained from ECACC (ECACC number 91072201).

Cells were split using 0.25% trypsin/EDTA (Sigma) and maintained in McCoy's 5a medium containing 10% Foetal Bovine Serum, 2mM Glutamine, 100U Penicillin, 0.1mg Streptomycin (Sigma) at 37°C, 5% $C0_2$.

Other cell lines include HCT116

Experiments

Effect of Mycograb (RTM) on HT29 cells

The cell lines were split and cells the counted. Cells were added to 12- or 96- well flat-bottomed tissue culture plates. In the case of the 12- well plates, 1ml of $4X10^4$ cells/ml or $4X10^5$ cells/ml were added to each well plus 1ml of complete McCoy's 5a medium. In the case of the 96- well plate, 100µl of $4X10^4$ cells/ml or $4X10^5$ cells/ml were added followed by a further 100µl of complete McCoy's 5a medium was added to each well. The plates were then incubated overnight at 37°C, 5% $C0_2$. The next day, the cells were observed under a phase contrast microscopy to ensure they had adhered to the plates and the supernatant medium removed by aspiration. Fresh complete RPMI medium containing two-fold increasing concentrations of Mycograb (RTM) (1.5 - 200µg/ml) or medium alone was then added to the wells. The plate was returned to the incubator for 48 hours following which the cell titre blue assay was carried out or viable counts were completed.

Effect of anti-cancer agents 5FU and folinic acid and Oxaliplatin on HT29 cells

The cell lines were split and the cells counted. 100µl of $4X10^4$ cells/ml or $4X10^5$ cells/ml were added to 96 well flat bottomed tissue culture plates a further 100µl of complete McCoy's 5a medium was added to the plate. The plates were then incubated overnight at 37°C, 5% $C0_2$. The next day, the cells were observed under phase contrast microscopy to ensure they had adhered to the plates and the supernatant medium removed by aspiration. 100µl of fresh complete RPMI medium containing increasing two fold concentrations of study drug (5-FU 4.5-2400µg/ml plus 1mg/ml Folinic acid or Oxaliplatin 1-500µg/ml) or medium alone (Media + 2.5% DMSO for 5-FU control) was then added to the wells. The plates were returned to the

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Incubator for 48 hours following which cell titre blue assays were carried out.

Effect of Mycograb (RTM) in combination with anti-cancer agents 5FU and folinic acid or Oxaliplatin on HT29 cells

The cell lines were split and the cells counted. 100µl of $4X10^4$ cells/ml or $4X10^5$ cells/ml were added to 96 well flat bottomed tissue culture plates a further 100µl of complete McCoy's 5a medium was added to the plate. The plates were then incubated overnight at 37°C, 5% $C0_2$. The next day, the cells were observed under phase contrast microscopy to ensure they had adhered to the plates and the supernatant medium removed by aspiration. 100µl of fresh complete RPMI medium was added to the plates and a checkerboard of Mycograb (RTM) versus study drug ((5-FU 4.5-2400µg/ml plus 1mg/ml Folinic acid or Oxaliplatin 1-500µg/ml) or medium alone (Medium + 2.5% DMSO for 5-FU control)) was set out as outlined in Table 16 to give a total volume of 200µl per well.

Effect of Mycograb (RTM) in combination with anti-cancer agents 5FU and folinic acid and Oxaliplatin on HT29 cells

The cell lines were split and the cells counted. 100µl of $4X10^4$ cells/ml or $4X10^5$ cells/ml were added to 96 well flat bottomed tissue culture plates a further 100µl of complete McCoy's 5a medium was added to the plate. The plates were then incubated overnight at 37°C, 5% $C0_2$. The next day, the cells were observed under phase contrast microscopy to ensure they had adhered to the plates and the supernatant medium removed by aspiration. 100µl of fresh complete RPMI medium was added to the plates and a checkerboard of Mycograb (RTM) versus study drug ((5-FU:Folinic acid:Oxaliplatin at a ratio of 3:1:0.42) or medium alone (Medium + 2.5% DMSO for 5-FU control)) was set out as for the 5-FU checkerboard outlined in Table 16 to give a total volume of 200µl per well.

Results

Effect of Mycograb (RTM) on HT29 cells

With cell line HT29 Mycograb (RTM) on its own at 125µg/ml demonstrated a 50% reduction in cell viability.

Effect of anti-cancer agents 5FU or Oxaliplatin alone and Mycograb (RTM) in combination with anti-cancer agents 5FU or Oxaliplatin on HT29 cells

5-FU:

The IC_{50} for 5-Fluorouracil was 150µg/ml. There was clear evidence of synergy between 5-FU and Mycograb (RTM) at a range of drug concentrations see Tables 17-20.

Oxaliplatin:

The IC_{50} for Oxaliplatin was 16µg/ml. There was some evidence of synergy between Oxaliplatin and Mycograb (RTM) at a range of drug concentrations, Table 18.

Effect of Mycograb (RTM) in combination with anti-cancer agents 5FU and Oxaliplatin on HT29 cells

The IC_{50} of LV/5FU/Ox was 25/75/10.5µg/ml. There was some evidence of synergy between 5-FU and Oxaliplatin and Mycograb (RTM) at a range of drug concentrations see Tables 22-24.

Summary

The results show that the antibody gave evidence of synergy with 5-FU and Folinic acid or Oxaliplatin and some evidence of synergy with the four drug combination of with 5-FU and Folinic acid and Oxaliplatin with concentrations of greater then 75µg/ml 5-FU and greater than 10.5µg/ml Oxaliplatin. There was evidence of synergy with 5-FU and Oxaliplatin, Table 25 summarises the CI values at ED_{50}, ED_{75} and ED_{90}.

Conclusions

The data presented here demonstrates that Mycograb (RTM) antibody on its own can decrease the viability of a colon adenocarcinoma cell line. There was synergy between anti-cancer agents, including 5-Fluorouracil and Oxaliplatin and the anti-HSP 90 antibody in a colon adenocarcinoma cell line. The data also demonstrates synergy between 5-Fluorouracil

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and Oxaliplatin with the anti-HSP 90 antibody in a colon adenocarcinoma cell line.

Table 1 **Checkerboard of Mycograb (MG) (µg/ml) versus Doxorubicin (DR) (µg/ml)**

	1	2	3	4	5	6	7	8	9	10	11	12
A	DR 150 MG 50	DR 75 MG 50	DR 37 MG 50	DR 18 5 MG 50	DR 9 MG 50	DR 4 5 MG 50	DR 2.3 MG 50	DR 1 12 MG 50	DR 0 55 MG 50	DR 0.27 MG 50	MG 50	Media no cells
B	DR 150 MG 25	DR 75 MG 25	DR 37 MG 25	DR 18.5 MG 25	DR 9 MG 25	DR 4 5 MG 25	DR 2.3 MG 25	DR 1 12 MG 25	DR 0 55 MG 25	DR 0.27 MG 25	MG 25	Media no cells
C	DR 150 MG 12 5	DR 75 MG 12.5	DR 37 MG 12.5	DR 18 5 MG 12.5	DR 9 MG 12.5	DR 4.5 MG 12.5	DR 2.3 MG 12.5	DR 1 12 MG 12 5	DR 0.55 MG 12.5	DR 0 27 MG 12 5	MG 12 5	Media no cells
D	DR 150 MG 6.25	DR 75 MG 6.25	DR 37 MG 6.25	DR 18.5 MG 6 25	DR 9 MG 6.25	DR 4.5 MG 6 25	DR 2.3 MG 6 25	DR 1 12 MG 6.25	DR 0.55 MG 6.25	DR 0.27 MG 6 25	MG 6 25	Media no cells
E	DR 150 MG 3	DR 75 MG 3	DR 37 MG 3	DR 18.5 MG 3	DR 9 MG 3	DR 4.5 MG 3	DR 2 3 MG 3	DR 1 12 MG 3	DR 0.55 MG 3	DR 0 27 MG 3	MG 3	Media no cells
F	DR 150 MG 1 5	DR 75 MG 1 5	DR 37 MG 1 5	DR 18.5 MG 1.5	DR 9 MG 1 5	DR 4 5 MG 1 5	DR 2 3 MG 1 5	DR 1 12 MG 1 5	DR 0 55 MG 1 5	DR 0.27 MG 1 5	MG 1.5	Media no cells
G	DR 150 MG 0 75	DR 75 MG 0 75	DR 37 MG 0 75	DR 18 5 MG 0 75	DR 9 MG 0 75	DR 4 5 MG 0 75	DR 2.3 MG 0 75	DR 1 12 MG 0 75	DR 0.55 MG 0 75	DR 0.27 MG 0 75	MG 0 75	Media no cells
H	DR 150	DR 75	DR 37	DR 18.5	DR 9	DR 4.5	DR 2 3	DR 1 12	DR 0 55	DR 0 27	Media plus cells	Media no cells

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Table 2

Cisplatin	Mycograb	CI
12.5	25	0.012
25	50	0.024

Table 3

Mycograb	Docetaxel	CI
12.5	100	0.400
25	200	0.098
50	400	0.002

Table 4

Mycograb	Doxorubicin	CI
0.75	2.25	0.249
1.5	4.5	0.261
3	9	0.248
6	18	0.476
12.5	37.5	0.186
25	75	0.155
50	150	0.159

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Table 5

Mycograb	Daunorubicin	CI
0.75	0.75	0.189
1.5	1.5	0.257
3	3	0.512
6	6	0.676
12.5	12.5	0.469
25	25	0.082
50	50	0.176

Table 6

Doxorubicin	Mycograb	CI
0.55	0.75	0.573
1.12	1.5	0.541
2.25	3	0.824
4.5	6	0.254
9	12	0.507
18.5	25	0.538
37	50	1.033

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Table 7

Mycograb	Daunorubicin	CI
0.75	0.75	1.153
1.5	1.5	1.300
3	3	1.557
6	6	0.763
12.5	12.5	0.367
25	25	1.014

Table 8

Mycograb	Herceptin	CI
0.75	0.75	0.007
1.5	1.5	0.008
3	3	0.005
6	6	0.034
12.5	12.5	0.025
25	25	0.170

Table 9 **Checkerboard of Mycograb (MG) (µg/ml) versus Doxorubicin (DR) (µg/ml)**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Media with cells	DR 0 27	DR 0.55	DR 1 12	DR 2 3	DR 4.5	DR 9	DR 18.5	DR 37	DR 75	DR 150	Media no cells
B	MG 0 75	DR 0 27 MG 0 75	DR 0 55 MG 0 75	DR 1 12 MG 0 75	DR 2 3 MG 0 75	DR 4 5 MG 0 75	DR 9 MG 0 75	DR 18 5 MG 0 75	DR 37 MG 0 75	DR 75 MG 0 75	DR 150 MG 0 75	Media no cells
C	MG 1 5	DR 0.27 MG 1.5	DR 0.55 MG 1.5	DR 1 12 MG 1.5	DR 2.3 MG 1.5	DR 4 5 MG 1.5	DR 9 MG 1 5	DR 18 5 MG 1 5	DR 37 MG 1 5	DR 75 MG 1 5	DR 150 MG 1 5	Media no cells
D	MG 3	DR 0 27 MG 3	DR 0 55 MG 3	DR 1 12 MG 3	DR 2 3 MG 3	DR 4 5 MG 3	DR 9 MG 3	DR 18 5 MG 3	DR 37 MG 3	DR 75 MG 3	DR 150 MG 3	Media no cells
E	MG 6.25	DR 0.27 MG 6.25	DR 0 55 MG 6.25	DR 1 12 MG 6.25	DR 2 3 MG 6.25	DR 4 5 MG 6.25	DR 9 MG 6.25	DR 18 5 MG 6 25	DR 37 MG 6 25	DR 75 MG 6.25	DR 150 MG 6.25	Media no cells
F	MG 12.5	DR 0 27 MG 12.5	DR 0 55 MG 12.5	DR 1 12 MG 12 5	DR 2.3 MG 12.5	DR 4 5 MG 12.5	DR 9 MG 12 5	DR 18 5 MG 12 5	DR 37 MG 12 5	DR 75 MG 12.5	DR 150 MG 12.5	Media no cells
G	MG 25	DR 0.27 MG 25	DR 0 55 MG 25	DR 1 12 MG 25	DR 2 3 MG 25	DR 4 5 MG 25	DR 9 MG 25	DR 18 5 MG 25	DR 37 MG 25	DR 75 MG 25	DR 150 MG 25	Media no cells
H	MG 50	DR 0 27 MG 50	DR 0 55 MG 50	DR 1 12 MG 50	DR 2 3 MG 50	DR 4 5 MG 50	DR 9 MG 50	DR 18 5 MG 50	DR 37 MG 50	DR 75 MG 50	DR 150 MG 50	Media no cells

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Table 10

Imatinib (µg/ml)	Mycograb (µg/ml)	CI
1	0.75	0.033
2	1.5	0.069
4	3	0.114
8	6	0.218
16	12.5	0.366
32	25	0.558
64	50	0.799

Table 11

Daunorubicin (µg/ml)	Mycograb (µg/ml)	CI
0.75	1.5	0.696
1.5	3	0.872

Table 12

Docetaxel (µg/ml)	Mycograb (µg/ml)	CI
1.5	1.5	0.001
3	3	0.008
6	6	0.707
12.5	12.5	0.051
25	25	0.013
50	50	0.003
100	100	0.004

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Table 13

Paclitaxel (µg/ml)	Mycograb (µg/ml)	CI
1	1.5	0.064
2	3	0.145
4	6	0.011
8	12.5	0.05
16	25	0.04
32	50	0.06
64	100	0.077

Table 14

Hydroxyurea (µg/ml)	Mycograb (µg/ml)	CI
0.3	0.75	0.798
0.6	0.75	0.694

Table 15

Imatinib (µg/ml)	Mycograb (µg/ml)	CI
1	0.375	0.131
2	1.5	0.093
4	3	0.016
8	6	0.001
16	12.5	0.002
32	25	0.327

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Table 16
Checkerboard of Mycograb (RTM) (MG in µg/ml) versus 5-Fluorouracil (5FU in µg/ml)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Media 2.5% DMSO	5FU 4.5	5FU 9	5FU 18.5	5FU 37	5FU 75	5FU 150	5FU 300	5FU 600	5FU 1200	5FU 2400	Media only, no cells
B	2.5% DMSO MG 3	5FU 4.5 MG 3	5FU 9 MG 3	5FU 18.5 MG 3	5FU 37 MG 3	5FU 75 MG 3	5FU 150 MG 3	5FU 300 MG 3	5FU 600 MG 3	5FU 1200 MG 3	5FU 2400 MG 3	Media only, no cells
C	2.5% DMSO MG 6	5FU 4.5 MG 6	5FU 9 MG 6	5FU 18.5 MG 6	5FU 37 MG 6	5FU 75 MG 6	5FU 150 MG 6	5FU 300 MG 6	5FU 600 MG 6	5FU 1200 MG 6	5FU 2400 MG 6	Media only, no cells
D	2.5% DMSO MG 12.5	5FU 4.5 MG 12.5	5FU 9 MG 12.5	5FU v MG 12.5	5FU 37 MG 12.5	5FU 75 MG 12.5	5FU 150 MG 12.5	5FU 300 MG 12.5	5FU 600 MG 12.5	5FU 1200 MG 12.5	5FU 2400 MG 12.5	Media only, no cells
E	2.5% DMSO MG 25	5FU 4.5 MG 25	5FU 9 MG 25	5FU 18.5 MG 25	5FU 37 MG 25	5FU 75 MG 25	5FU 150 MG 25	5FU 300 MG 25	5FU 600 MG 25	5FU 1200 MG 25	5FU 2400 MG 25	Media only, no cells
F	2.5% DMSO MG 50	5FU 4.5 MG 50	5FU 9 MG 50	5FU 18.5 MG 50	5FU 37 MG 50	5FU 75 MG 50	5FU 150 MG 50	5FU 300 MG 50	5FU 600 MG 50	5FU 1200 MG 50	5FU 2400 MG 50	Media only, no cells
G	2.5% DMSO MG 100	5FU 4.5 MG 100	5FU 9 MG 100	5FU 18.5 MG 100	5FU 37 MG 100	5FU 75 MG 100	5FU 150 MG 100	5FU 300 MG 100	5FU 600 MG 100	5FU 1200 MG 100	5FU 2400 MG 100	Media only, no cells
H	2.5% DMSO MG 200	5FU 4.5 MG 200	5FU 9 MG 200	5FU 18.5 MG 200	5FU 37 MG 200	5FU 75 MG 200	5FU 150 MG 200	5FU 300 MG 200	5FU 600 MG 200	5FU 1200 MG 200	5FU 2400 MG 200	Media only, no cells

Table 17
5-Fluorouracil and Mycograb at a ratio of 0.37:1

5-FU (ug/ml)	Mycograb (ug/ml)	CI
4.5	12.5	0.001
9	25	0.004
18.5	50	0.008
37	100	0.049

Table 18
5-Fluorouracil and Mycograb at a ratio of 0.75:1

5-FU (ug/ml)	Mycograb (ug/ml)	CI
4.5	6	0.184
9	12.5	0.295

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Table 19
5-Fluorouracil and Mycograb at a ratio of 12:1

5-FU (ug/ml)	Mycograb (ug/ml)	CI
37	3	0.557
75	6	0.228
150	12.5	0.198
300	25	0.540
600	50	0.250
1200	100	0.478
2400	100	0.212

Table 20
5-Fluorouracil and Mycograb at a ratio of 50:1

5-FU (ug/ml)	Mycograb (ug/ml)	CI
75	1.5	0.004
150	3	0.342
300	6	0.482

Table 21
Oxaliplatin and Mycograb at a ratio of 1.25:1

Oxaliplatin (ug/ml)	Mycograb (ug/ml)	CI
7.5	6	0.378
15.5	12.5	0.182
31	25	0.153
62.5	50	0.046
125	100	0.616
250	200	0.185

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Table 22
5-Fluorouracil, Oxaliplatin and Mycograb at a ratio of 3:1:0.42

5-FU	Mycograb	Oxaliplatin	CI
(ug/ml)	(ug/ml)	(ug/ml)	
9	3	1.3	0.053
18.5	6	2.6	0.109
37	12.5	5.25	0.239
75	25	10.5	3.120

Table 23
5-Fluorouracil, Oxaliplatin and Mycograb at a ratio of 3:2:0.42

5-FU	Mycograb	Oxaliplatin	CI
(ug/ml)	(ug/ml)	(ug/ml)	
9	6	1.3	0.074
18.5	12.5	2.6	0.152
37	25	5.25	0.405
75	50	10.5	1.519

Table 24
5-Fluorouracil, Oxaliplatin and Mycograb at a ratio of 3:0.5:0.42

5-FU	Mycograb	Oxaliplatin	CI
(ug/ml)	(ug/ml)	(ug/ml)	
9	1.5	1.3	0.043
18.5	3	2.6	0.089
37	6	5.25	0.184
75	12.5	10.5	2.202

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Table 25

Drug (ratio)	Combination Index Values at				
	ED50	ED75	ED90	Dm	r
5-FU	N/A	N/A	N/A	297.1493	0.95571
Mycograb	N/A	N/A	N/A	96.46543	0.87307
Oxaliplatin	N/A	N/A	N/A	27.77133	0.94124
5-FU/Mycograb/ Oxaliplatin	0.77008	0.11419	0.02256	64.92005	0.87996
(3 : 1 : 0.42)					
5-FU/Mycograb/ Oxaliplatin	0.85085	0.16415	0.03924	55.54798	0.97163
(1.5 : 1 : 0.21)					
5-FU/Mycograb/ Oxaliplatin	0.62267	0.09916	0.02224	61.08042	0.9142
(6 : 1 :0.85)					

CLAIMS

1. The use of:

   (I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

   (II) at least one anti-cancer agent selected from the group consisting of: Doxorubicin, Daunorubicin, Epirubicin, Herceptin, Docetaxel, and Cisplatin,

in a method of manufacture of a medicament for the treatment of cancer.

2. A combined preparation comprising:

   (i) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

   (II) at least one anti-cancer agent selected from the group consisting of: Doxorubicin, Daunorubicin, Epirubicin, Herceptin, Docetaxel, and Cisplatin,

for simultaneous, separate or sequential use in the treatment of cancer.

3. The use or combined preparation according to either of claims 1 or 2, wherein said antibody or antigen binding fragment thereof is specific for the epitope displayed by the peptide having the sequence of SEQ ID NO: 1.

4. The use or combined preparation according to any of claims 1-3, wherein said antibody comprises the sequence of SEQ ID NO: 2.

5. The use or combined preparation according to any of claims 1-4, wherein said cancer is selected from the group consisting of: fibrosarcoma, breast, prostate, melanoma, leukemia, lymphomas, colon, testicular germ cell, pancreatic, ovarian, endometrial, thyroid, and lung.

6. A method of treatment of cancer comprising administering a therapeutically effective quantity of:

   (I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(II) at least one anti-cancer agent selected from the group consisting of: Doxorubicin, Daunorubicin, Epirubicin, Herceptin, Docetaxel, and Cisplatin,

to a patient in need of same.

7. The use of:

(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90;

in a method of manufacture of a medicament for the treatment of leukaemia.

8. The use of:

(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(II) at least one anti-cancer agent selected from the group consisting of: Imatinib, Paclitaxel, Docetaxel, Daunorubicin, Doxorubicin, and Hydroxyurea,

in a method of manufacture of a medicament for the treatment of leukaemia.

9. A combined preparation comprising:

(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(II) at least one anti-cancer agent selected from the group consisting of: Imatinib, Paclitaxel, Docetaxel, Daunorubicin, Doxorubicin, and Hydroxyurea,

for simultaneous, separate or sequential use in the treatment of leukaemia.

10. The use or combined preparation according to any of claims 7-9, wherein said antibody or antigen binding fragment thereof is specific for the epitope displayed by the peptide having the sequence of SEQ ID NO: 1.

11. The use or combined preparation according to any of claims 7-10, wherein said antibody comprises the sequence of SEQ ID NO: 2.

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12. The use or combined preparation according to any of claims 7-11, wherein said leukaemia is selected from the group consisting of: acute myeloblastic leukaemia, acute lymphoblastic leukaemia, chronic myeloid leukaemia, and chronic lymphocytic leukaemia.

13. The use or combined preparation according to any of claims 7-12, wherein said at least one anti-cancer agent is Imatinib.

14. The use or combined preparation according to any of claims 7-13, wherein said leukaemia is chronic myeloid leukaemia or acute lymphoid leukaemia.

15. The use or combined preparation according to claim 14, wherein said leukaemia is characterized by cells which are Philadelphia chromosome positive, or cells which are Philadelphia chromosome negative.

16. The use or combined preparation according to claim 14, wherein said anti-cancer agent is Imatinib, and said leukaemia is characterized by cells which are Philadelphia chromosome positive.

17. The use or combined preparation according to claim 14, wherein said anti-cancer agent is Imatinib, and said leukaemia is characterized by cells which are Philadelphia chromosome negative.

18. The use or combined preparation according to any of claims 7-17, wherein said leukaemia is characterized by cells which are Imatinib resistant.

19. A method of treatment of leukaemia comprising administering a therapeutically effective quantity of:

(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(II) at least one anti-cancer agent selected from the group consisting of: Imatinib, Paclitaxel, Docetaxel, Daunorubicin, Doxorubicin, and Hydroxyurea,

to a patient in need of same.

20. The method according to claim 19, wherein said leukaemia is chronic myeloid leukaemia, and said at least one anti-cancer agent is imatinib.

21. The use of:

(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(II) at least one anti-cancer agent selected from the group consisting of: 5-fluorouracil, oxaliplatin, irinotecan and raltitrexed,

in a method of manufacture of a medicament for the treatment of cancer.

22. A combined preparation comprising:

(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(II) at least one anti-cancer agent selected from the group consisting of:, 5-fluorouracil, oxaliplatin, irinotecan and raltitrexed,

for simultaneous, separate or sequential use in the treatment of cancer.

23. The use or combined preparation according to either of claims 21 or 22, wherein said antibody or antigen binding fragment thereof is specific for the epitope displayed by the peptide having the sequence of SEQ ID NO: 1.

24. The use or combined preparation according to any of claims 21-23, wherein said antibody comprises the sequence of SEQ ID NO: 2.

25. The use or combined preparation according to any of claims 21-24 wherein said cancer is selected from the group consisting of: fibrosarcoma, adenocarcinoma, breast, prostate, melanoma, leukaemia, lymphomas, colon, colorectal, testicular germ cell, pancreatic, ovarian, endometrial, thyroid, and lung.

26. The use or combined preparation according to claim 25 wherein said cancer is colorectal cancer or adenocarcinoma.

27. A method of treatment of cancer comprising administering a therapeutically effective quantity of:

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(I) an antibody or an antigen binding fragment thereof specific for at least one epitope of Hsp90; and

(II) at least one anti-cancer agent selected from the group consisting of: 5-fluorouracil, oxaliplatin, irinotecan and raltitrexed,

to a patient in need of same.

28. The use, combined preparation or method according to any of claims 21 to 27 wherein the anti-cancer agent is 5-fluorouracil and further comprises folinic acid (leucovorin).

29. The use, combined preparation or method according to claim 28 wherein the anti-cancer agent comprises 5-fluorouracil, folinic acid (leucovorin) and oxaliplatin.

30. The method according to any of claims 6, 19, 20, 27, 28 or 29 wherein said composition or combined preparation is administered orally.

31. The use, combined preparation or method according to any of claims 1-30, wherein said antibody or antigen binding fragment is labelled with a detectable label.

32. The use, combined preparation or method according to any of claims 1-31, wherein said antibody or antigen binding fragment is conjugated with an effector molecule.

33. The invention as substantially hereinbefore described with reference to the examples.

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SEQUENCE LISTING

<110> NeuTec Pharma plc
Burnie, James P
Matthews, Ruth C
Carter, Tracey

<120> Treatment of Cancer

<130> WA/M101423WO

<150> US 60/654,458
<151> 2005-02-22

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<151> 2004-09-30

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<151> 2004-07-02

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55

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    130                 135                 140

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Cys Arg Ala Ser Ser Gly Ile Ser Arg Tyr Leu Ala Trp Tyr Gln Gln
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Gln Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu
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Phe Thr Leu Thr Ile Asn Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
    210                 215                 220

Tyr Cys Gln His Leu Asn Ser Tyr Pro Leu Thr Phe Gly Gly Gly Thr
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Lys Val Asp Ile Lys Arg Ala Ala
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INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/GB2005/002545

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/395 C07K16/18

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)
IPC 7 C07K 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, WPI Data, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MALONEY ALISON ET AL: "HSP90 as a new therapeutic target for cancer therapy: the story unfolds." EXPERT OPINION ON BIOLOGICAL THERAPY. JAN 2002, vol. 2, no. 1, January 2002 (2002-01), pages 3-24, XP009053600 ISSN: 1471-2598 the whole document -/--	1-33

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

"E" earlier 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)

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 September 2005	21/09/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hermann, P

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/GB2005/002545

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	SCHWARTZ JOSEPH ET AL: "Novel targeted and immunotherapeutic strategies in chronic myeloid leukemia." SEMINARS IN HEMATOLOGY. JAN 2003, vol. 40, no. 1, January 2003 (2003-01), pages 87-96, XP009053575 ISSN: 0037-1963 cited in the application the whole document	1-33
X	BANERJI UDAI ET AL: "The clinical applications of heat shock protein inhibitors in cancer - present and future." CURRENT CANCER DRUG TARGETS. OCT 2003, vol. 3, no. 5, October 2003 (2003-10), pages 385-390, XP009053559 ISSN: 1568-0096 cited in the application the whole document	1-33
X	WO 00/61578 A (SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH; ROSEN, NEAL; KUDUK, SCO) 19 October 2000 (2000-10-19) the whole document	1-33
A	WO 01/76627 A (NEUTEC PHARMA PLC; BURNIE, JAMES, PETER) 18 October 2001 (2001-10-18) the whole document	1-33
A	WHITESELL L ET AL: "Inhibition of heat shock protein HSP90-pp60-v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 91, no. 18, 16 August 1994 (1994-08-16), pages 8324-8328, XP002175506 ISSN: 0027-8424 the whole document	1-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2005/002545

Box 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. [X] Claims Nos.: 33
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

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).

Box 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 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.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Claims Nos.: 33

Although claims 6, 19, 20, 27-32 are directed to a method of treatment of the human/animal body by therapy (Rule 39.1(iv) PCT), the search has been carried out and based on the alleged effects of the compound/composition.

Claim 33 does not contain any characteristic features defining the subject-matter for which protection is sought, therefore claim 33 lacks clarity (Article 6 PCT) to such an extent that no meaningful search could be performed as regards its subject-matter.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int nal Application No
PCT/GB2005/002545

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0061578	A	19-10-2000	AU	769235 B2	22-01-2004
			AU	4223500 A	14-11-2000
			CA	2370007 A1	19-10-2000
			EP	1169319 A1	09-01-2002
			JP	2002541255 T	03-12-2002
WO 0176627	A	18-10-2001	AU	4089001 A	23-10-2001
			BR	0109846 A	03-06-2003
			CA	2401836 A1	18-10-2001
			CN	1420786 A	28-05-2003
			EP	1267925 A1	02-01-2003
			JP	2003530357 T	14-10-2003
			NO	20024815 A	02-12-2002
			NZ	520899 A	24-03-2005
			PL	358394 A1	09-08-2004
			US	2003180285 A1	25-09-2003

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<110> NeuTec Pharma plc
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Carter, Tracey

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Lys Val Asp Ile Lys Arg Ala Ala
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TRATAMIENTO DE CÁNCER

CAMPO DE LA INVENCION

La presente invención se refiere a medicamentos y preparados novedosos que comprenden agentes farmacéuticos efectivos junto con un anticuerpo anti-Hsp 90 los cuales juntos proveen una eficacia incrementada en el tratamiento de cánceres, incluyendo cáncer colorectal. Otros aspectos de la invención se refieren al tratamiento de leucemias.

ANTECEDENTES DE LA INVENCION

Terapia para cáncer y tratamiento de leucemia

Un primer aspecto de la presente invención se refiere a medicamentos y preparados novedosos que comprenden agentes anti-cáncer efectivos junto con un anticuerpo anti-Hsp90 los cuales juntos proveen una eficacia incrementada en el tratamiento de cáncer.

En años recientes han surgido miembros de la familia de proteínas de proteínas de choque térmico (Hsp) que tienen una función importante en oncogénesis y muerte celular. En efecto, se han identificado a las proteínas de choque térmico como objetivos potenciales para terapia de cáncer durante muchos años (Whitesell *et al.*, PNAS USA, 30

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de agosto de 1994, 91(18):8324-8; PMID: 8078881), y miembros de la familia de ansamicina (anteriormente conocidas como inhibidores de tirosina cinasa) han sido sugeridos como útiles para efectuar la terapia contra cáncer (Neckers L *et al.*, Invest New Drugs, 1999, 17(4): 361-73; PMID: 10759403; Schulte TW *et al.*, Cáncer Chemother Pharmacol., 1998, 42(4): 273-9; PMID: 9744771).

Una proteína de choque térmico, Hsp90, ha sido implicada como involucrada en carcinoma de tejido mamario, de próstata, melanoma, leucemias y linfomas, cáncer de colon y pulmón (Banerji U *et al.*, Curr Cancer Drug Targets, octubre de 2003; 3(5): 385-90; PMID: 14529390), así como en carcinomas de la tiroides. El papel de Hsp90 es asegurar el plegamiento correcto de las "proteínas cliente" las cuales están implicadas en una amplia variedad de procesos celulares, por ejemplo transducción de señal. Las proteínas cliente de Hsp90 incluyen factores de transcripción tales como p53 mutante y el factor 1α inducible por hipoxia, y cinasas solubles incluyendo v-Src, Akt, Raf-1, y Bcr-Abl. Hsp90 se expresa en forma constitutiva a niveles 2 a 10 veces más altos en células de tumor que en células normales, lo que sugiere que ésta podría ser importante para el crecimiento/supervivencia de células de tumor (Schwartz, J., *et al.*, 2003, Semin. Hematol 40: p87-96). Debido a que la unión de las proteínas cliente a Hsp90

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puede regular su conformación, estabilidad y destino en la célula, Hsp90 puede tener un impacto importante sobre las rutas que regulan el resultado celular, incluyendo crecimiento, división, diferenciación, movimiento y muerte celular (Workman, P., Cáncer Lett. 8 de abril de 2004; 206(2):149-57; PMID: 15013520). La función de amplio alcance para Hsp90 en procesos celulares significa que la proteína actualmente se considera como un posible objetivo para el desarrollo de fármacos terapéuticos. Los inhibidores de Hsp90, al interactuar específicamente con un solo objetivo molecular, ocasionan la desestabilización y degradación eventual de las proteínas cliente de Hsp90.

Un segundo aspecto de la presente invención se refiere a medicamentos y preparados novedosos que comprenden agentes anti-cáncer efectivos junto con un anticuerpo anti-Hsp90 los cuales juntos proveen una eficacia incrementada en el tratamiento de leucemia.

Leucemia es un cáncer que afecta la médula ósea. En personas con leucemia, la médula ósea produce números grandes de linfocitos anormales. Los linfocitos anormales se acumulan en la médula ósea, de modo tal que la médula ósea no puede elaborar suficientes eritrocitos, linfocitos y plaquetas normales.

Los tipos diferentes de leucemia se pueden clasificar por su velocidad de desarrollo (aguda o

66

crónica), y por el tipo de leucocito afectado, (células mieloides o linfoides). Los leucocitos mieloides son la primera línea de defensa del sistema inmune contra la infección y se encuentran principalmente en la sangre, en donde estos engullen y aniquilan organismos extraños. Los leucocitos linfoides se encuentran en los nódulos linfáticos y en la sangre.

Los cuatro tipos más comunes de leucemia incluyen leucemia linfoide crónica (linfocítica) (CLL), leucemia mieloide aguda (mieloblástica) (AML), leucemia linfoide aguda (linfoblástica) (ALL), y leucemia mieloide crónica (CML).

CLL también es un cáncer de los linfocitos pero se desarrolla de manera más lenta que ALL. Esta enfermedad es el tipo más común de leucemia que afecta a los adultos, y es muy rara en niños.

AML es un cáncer que afecta principalmente las células mieloides conocidas como granulocitos. Esta crea demasiados mieloblastos que pueden bloquear los vasos sanguíneos, y no suficientes células mieloides maduras. Esta enfermedad se presenta principalmente en adultos pero también puede afectar a niños.

CML, (también llamada leucemia granulocítica crónica) típicamente es un cáncer de células neutrófilas de avance muy lento, el cual es raro en niños y comúnmente

afecta a adultos de género masculino más que a los adultos de género femenino. CML por lo general se diagnostica fácilmente debido a que las células leucémicas de más del 95% de los pacientes tienen una anormalidad citogenética distintiva, el cromosoma Filadelfia (Ph1). (Kurzrock, R. *et al.* 2003, Ann. Intern. Med. 138(10): p819-30, PMID: 12755554; Goldman, J.M. y Melo, J.V., 2003, N. Engl. J. Med. 349(15): p1451-64, PMID: 14534339). El Ph1 resulta de una translocación recíproca entre los brazos largos de los cromosomas 9 y 22 y se puede demostrar en todos los precursores hematopoyéticos (Deininger, M.W. *et al.* 2000, Blood 96 (10):p3343-56, PMID: 11071626). Esta translocación da como resultado la transferencia del oncogen Abelson (abl) en el cromosoma 9, hacia un área del cromosoma 22 denominado la región del grupo de punto de ruptura (BCR) (Deininger, M.W. *et al.* 2000, Blood 96(10):p3343-56, PMID: 11071626). Esto a su vez da como resultado un gen BCR/ABL fusionado que codifica para un ARNm quimérico de 8.5 kb. El gen BCR/ABL es un oncogen que es suficiente para producir enfermedad tipo CML en ratones. El transcrito del oncogen BCR/ABL se traduce para producir una proteína de 210 kDa o 190 kDa. La proteína Bcr-Abl es una proteína tirosina cinasa anormal que ocasiona la mielopoyesis desordenada encontrada en CML. CML avanza a través de etapas clínicas distintas denominadas fase crónica, fase acelerada, y

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crisis blástica. El oncogen BCR/ABL se expresa en todas las etapas, pero la crisis blástica se caracteriza por múltiples cambios genéticos y moleculares adicionales (Gorre, M.E., *et al.* 2002, Blood, 100(8): p3041-3044).

CML Ph1-negativa es una enfermedad rara que se caracteriza por las características clínicas de CML sin evidencia citogenética o molecular (RT-PCR) de la translocación t(9;22)(q34;q11) que resulta en el ARNm de fusión de Bcr-Abl. CML Ph1-negativa es una entidad pobremente definida que se distingue de manera menos clara de otros síndromes mielo-proliferativos. Aunque alguna vez se pensó que explica el 5-10% de todas las CML clínicas, con la accesibilidad rutinaria del análisis RT-PCR para el transcrito Bcr-Abl, dicho número actualmente está por debajo del 5%. Como un aspecto interesante, algunos pacientes con esta entidad pueden resultar de una fusión alternativa a Abl. La fusión TEL(ETV6)-ABL, como resultado de t(9;12), ha sido demostrada en dos casos de CML Ph negativa. Los pacientes con CML Ph1-negativa generalmente tienen una respuesta más baja hacia el tratamiento y supervivencia más corta que los pacientes Ph1-positivos (Onida, F. *et al.* 2002: Cancer 95(8):p1673-84, PMID: 12365015).

ALL es un cáncer de linfocitos inmaduros, conocidos como linfoblastos. Esta enfermedad es el tipo más

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común de leucemia en niños jóvenes, normalmente entre las edades de 1 y 7 años y es bastante rara en adultos. ALL ocasiona que se elaboren muchos linfocitos anormales, los cuales superan en número los eritrocitos y plaquetas normales. Se ha implicado directamente una proteína Bcr-Abl de 185 kDa en el desarrollo de ALL.

Dos fármacos, geldanamicina (GA), y 17-alilamino, 17-desmetoxigeldanamicina (17-AAG) los cuales actúan como inhibidores de Hsp90, han demostrado actividad biológica y clínica prometedora en pruebas clínicas. En efecto, la proteína de fusión Bcr-Abl de 210 kDa ($p210^{Bcr-Abl}$) depende de su asociación con Hsp90 para su estabilidad, y el tratamiento de las células con GA o 17-AAG conduce a la destrucción rápida de $p210^{Bcr-Abl}$.

Un inhibidor de Hsp90 tal como 17-AAG en combinación con agentes citotóxicos convencionales u otros agentes novedosos, también podría ser terapéuticamente valioso para atacar oncogénesis de etapas múltiples (Workman P., Cancer Lett. 8 de abril de 2004; 206(2):149-57; PMID: 15013520). En células cancerosas, las cuales se caracterizan por inestabilidad genética, es posible que 17AAG, al bloquear la actividad de Hsp90, libere una variedad de mutaciones que juntas demuestran ser "sintéticamente letales" para el tumor. Las células normales, las cuales carecen de la inestabilidad genética

de las células tumorales, se ven relativamente inafectadas (Garber, K., 2002, Journal of the National Cancer Institute, Vol. 94, No. 22, p1666-1668). Un problema significativo con 17AAG es que el fármaco es demasiado tóxico para terapia prolongada, y por consiguiente existe la necesidad de un reemplazo no tóxico (Banerji *et al., supra*).

El mesilato de imatinib (Gleevec (RTM)) es un inhibidor de tirosina cinasa de molécula pequeña que tiene un mayor impacto sobre una enfermedad neoplásica como un agente individual. Diseñado originalmente como un inhibidor de la tirosina cinasa Bcr-Abl característica de los tumores malignos que portan la translocación 9;22 patogénica, imatinib ha demostrado ser moderadamente específico, y ha logrado un impacto importante en el tratamiento de leucemia mielógena crónica (CML) y ALL positiva en cuanto al cromosoma Filadelfia (Ph1+) (Krystal, GW, 2004, Leukemia Research 28S1:pS53-S59). Uno de los problemas asociados con el tratamiento de CML con imatinib, es la resistencia al fármaco como resultado de mutaciones en la tirosina cinasa Bcr-Abl. Como un aspecto importante, las células de CML que se han vuelto resistentes a imatinib *in vivo* conservan su dependencia en Hsp90 y por lo tanto permanecen sensibles a 17AAG.

Publicaciones recientes enseñan que Hsp90 de

tumor está presente completamente en complejos de chaperones múltiples que facilitan el avance del tumor maligno y que estos son objetivos atractivos para agentes terapéuticos contra el cáncer. En particular, se describe que Hsp90 en complejos de chaperones múltiples obtenidos a partir de células tumorales tiene una afinidad de unión 100 veces más alta para 17AAG que para Hsp90 proveniente de células normales (es decir, Hsp90 en su estado no complejado latente), lo que indica que en el complejo de chaperones múltiples éste puede desplegar epítopes (en particular epítopes cuaternarios) no desplegados por Hsp90 no complejado latente. El anticuerpo Mycograb (RTM) se puede unir a Hsp90 en su estado no complejado latente, y además en complejos de chaperones múltiples, sin ninguno de los efectos adversos sobre la cinética de unión.

El documento WO 01/76627 enseña composiciones para el tratamiento de infecciones fúngicas, las composiciones comprenden una combinación de (i) un agente anti-fúngico inhibidor de polieno o beta glucano sintetasa; y (ii) anticuerpos específicos contra Hsp90 fúngica, las composiciones son efectivas contra el hongo que ocasiona la infección a pesar que éste sea resistente al agente anti-fúngico *per se*.

SUMARIO DE LA INVENCION

De conformidad con un primer aspecto de la presente invención se provee el uso de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: doxorrubicina, daunorrubicina, epirrubicina, herceptina, docetaxel, y cisplatina,

en un método de fabricación de un medicamento para el tratamiento de cáncer.

Doxorrubicina es un agente antibiótico tipo antraciclina previamente reconocido como un agente anti-tumor.

Epirrubicina es un antibiótico tipo antraciclina sintético menos tóxico, también reconocido previamente como un agente anti-tumor.

Daunorrubicina es un fármaco anti-neoplásico utilizado en un número de campos terapéuticos, incluyendo como un agente anti-cáncer.

Herceptina (Trastuzumab) es un anticuerpo monoclonal utilizado para el tratamiento de cáncer de tejido mamario metastásico que sobre-expresa a la proteína

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HER2.

Docetaxel es un agente anti-cáncer reconocido, y es un inhibidor mitótico.

Cisplatina es un agente anti-cáncer reconocido, y comprende un complejo de platino.

Como se describe de manera más detallada en los resultados experimentales más adelante ("Experimentos A"), doxorrubicina y daunorrubicina son particularmente preferidos, y demuestran efectos sinergistas particularmente buenos con el anticuerpo anti-Hsp90. Herceptina también demuestra efectos sinergistas adecuados con el anticuerpo anti-Hsp90. También se observa sinergia con docetaxel y cisplatina cuando se combinan con anticuerpo anti-Hsp90. El sinergismo entre daunorrubicina y el anticuerpo es particularmente evidente con células positivas a receptor de estrógeno, y de esta manera los medicamentos y terapias que utilizan al anticuerpo y daunorrubicina pueden ser en particular para (o administrar a o para) células que tengan receptores de estrógeno.

Los experimentos ("Experimentos A") también demuestran que otros agentes anti-cáncer cuando se utilizan juntos con anticuerpo anti-Hsp90 pueden mostrar resultados indiferentes (paclitaxel) o antagonismo (imatinib). Esto confirma la naturaleza sorpresiva/inesperada del sinergismo logrado con los agentes anti-cáncer anteriores cuando se

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combinan con anticuerpo anti-Hsp90.

También se provee un preparado combinado que comprende:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: doxorrubicina, daunorrubicina, epirrubicina, herceptina, docetaxel, y cisplatina,

para el uso simultáneo, por separado o en secuencia en el tratamiento de cáncer.

También se provee un método de tratamiento de cáncer que comprende administrar una cantidad terapéuticamente efectiva de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: doxorrubicina, daunorrubicina, epirrubicina, herceptina, docetaxel, y cisplatina,

a un paciente en necesidad del mismo.

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DESCRIPCION DETALLADA DE LA INVENCION

Tal como se utiliza en la presente invención, el término "tratamiento" pretende tener un significado amplio a menos que se indique explícitamente lo contrario. Por lo tanto, mediante "tratamiento" o "terapia" se quiere decir cualquier tratamiento que esté diseñado para curar, aliviar, eliminar o aminorar los síntomas de, o evitar o reducir la posibilidad de contraer trastornos o disfunciones del cuerpo humano o animal. Por lo tanto, mediante el término "tratamiento" se quiere decir tanto tratamiento de condiciones patológicas, así como su profilaxis.

El anticuerpo o fragmento de unión a antígeno del mismo puede ser específico para el epítope desplegado por un péptido que comprende la secuencia de SEQ ID NO:1.

Como se discutió anteriormente, aunque los epítopes cuaternarios desplegados por Hsp90 en complejos de chaperón múltiple han sido sugeridos como objetivos apropiados para terapia, los experimentos (más adelante) muestran que de hecho un epítope lineal es un objetivo útil y efectivo para terapia.

Los anticuerpos, su fabricación y usos son bien conocidos y se describen en, por ejemplo, Harlow, E. y Lane, D., Antibodies: A Laboratory Manual, Cold Spring

Harbor Laboratory Press, Cold Spring Harbor; New York, 1999.

Los anticuerpos se pueden generar utilizando métodos estándar conocidos en la técnica. Los ejemplos de anticuerpos incluyen (pero no se limitan a) anticuerpos policlonales, monoclonales, quiméricos, de cadena individual, fragmentos Fab, fragmentos producidos por una genoteca de expresión de Fab, y fragmentos de unión a antígeno de anticuerpos.

Los anticuerpos se puede producir en una variedad de hospederos, por ejemplo cabras, conejos, ratas, ratones, humanos, y otros. Estos se pueden inmunizar mediante inyección con proteínas de tensión fúngicas, o cualquier fragmento u oligopéptido de las mismas que tenga propiedades inmunógenas. Dependiendo de la especie hospedera, se pueden utilizar diversos coadyuvantes para incrementar una respuesta inmunológica. Dichos coadyuvantes incluyen, pero no se limitan a, coadyuvante de Freund, geles minerales tales como hidróxido de aluminio, y sustancias tensoactivas tales como lisolecitina, polioles tipo plurónic, polianiones, péptidos, emulsiones oleosas, hemocianina de lapa, y dinitrofenol. Entre los coadyuvantes utilizados en humanos, son particularmente útiles BCG (Bacilo de Calmette-Guerin) y *Corynebacterium parvum*.

Los anticuerpos monoclonales para proteínas

fúngicas, o cualquier fragmento u oligopéptido de las mismas se pueden preparar utilizando cualquier técnica que provea la producción de moléculas de anticuerpo mediante líneas celulares continuas en cultivo. Estas incluyen, pero no se limitan a, la técnica de hibridoma, la técnica de hibridoma de célula B de humano, y la técnica de hibridoma de EBV (Koehler *et al.*, 1975, Nature, 256: 495-497; Kosbor *et al.*, 1983, Immunol. Today 4:72; Cote *et al.*, 1983, PNAS USA, 80:2026-2030; Cole *et al.*, 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss Inc., New York, pp. 77-96).

Además, se pueden utilizar técnicas desarrolladas para la producción de "anticuerpos quiméricos", el empalme de genes de anticuerpo de ratón con genes de anticuerpo de humano para obtener una molécula con especificidad de antígeno y actividad biológica apropiadas (Morrison *et al.*, 1984, PNAS USA, 81:6851-6855; Neuberger *et al.*, 1984, Nature, 312: 604-608; Takeda *et al.*, 1985, Nature, 314: 452-454). De manera alternativa, se pueden adaptar técnicas descritas para la producción de anticuerpos de cadena individual, utilizando métodos conocidos en la técnica, para producir anticuerpos de cadena individual específicos de proteína de tensión fúngica. Los anticuerpos con especificidad relacionada, pero de composición idiotípica distinta, se pueden generar mediante intercambio de cadena a partir de genotecas de inmunoglobulina de combinación

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aleatorias (Burton, D.R., 1991, PNAS USA, 88:11120-11123).

También se pueden producir anticuerpos induciendo la producción *in vivo* en la población de linfocitos o mediante tamizaje de genotecas de inmunoglobulina recombinante o paneles de reactivos de unión altamente específicos (Orlandi *et al.*, 1989, PNAS USA, 86:3833-3837; Winter, G. *et al.*, 1991, Nature, 349:293-299).

También se pueden generar fragmentos de unión a antígeno, por ejemplo los fragmentos F(ab')2 los cuales pueden ser producidos digiriendo con pepsina la molécula de anticuerpo y los fragmentos Fab los cuales se pueden generar reduciendo los puentes de disulfuro de los fragmentos F(ab')2. De manera alternativa, se pueden construir genotecas de expresión de Fab para permitir la identificación rápida y fácil de fragmentos Fab monoclonales con la especificidad deseada (Huse *et al.*, 1989, Science, 256: 1275-1281).

Se pueden utilizar variar pruebas inmunológicas para pruebas de tamizaje para identificar anticuerpos que tengan la especificidad deseada. En la técnica son bien conocidos numerosos protocolos para unión competitiva o pruebas inmuno-radiométricas utilizando anticuerpos ya sea policlonales o monoclonales con especificidades establecidas. Dichas pruebas inmunológicas típicamente implican la medición de formación de complejo entre la

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proteína de tensión fúngica o cualquier fragmento u oligopéptido de la misma, y su anticuerpo específico. Se puede utilizar una prueba inmunológica basada en monoclones, de dos sitios utilizando anticuerpos monoclonales específicos para dos epítopes de proteína de tensión fúngica no interferentes, pero también se puede utilizar una prueba de unión competitiva (Maddox *et al.*, 1983, J. Exp. Med., 158:1211-1216).

Por ejemplo, el anticuerpo utilizado en la composición o preparado combinado puede comprender la secuencia de SEQ ID NO:2.

El inventor de la presente ha descubierto que los cánceres que pueden ser tratados de manera útil incluyen fibrosarcomas y carcinomas que se seleccionan a partir del grupo que consiste de: carcinomas de tejido mamario, de próstata, melanoma, leucemia, linfomas, leucemia, de colon, de célula germinal testicular, de páncreas, ovárico, del endometrio, de tiroides, y de pulmón.

De conformidad con un segundo aspecto de la presente invención se provee el uso de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90;

en un método para fabricar un medicamento para el tratamiento de leucemia.

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También se provee el uso de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: imatinib, paclitaxel, docetaxel, daunorrubicina, doxorrubicina, e hidroxiurea,

en un método para fabricar un medicamento para el tratamiento de leucemia.

Imatinib, un derivado de 2-fenilaminopirimidina, es un antagonista de molécula pequeña con actividad contra proteína tirosina cinasas, y exhibe inhibición potente y específica de Bcr-Abl. Imatinib está indicado para el tratamiento de pacientes con CML en crisis blástica, fase acelerada, o en fase crónica después del fracaso de terapia con IFN.

Paclitaxel es un agente quimioterapéutico que se administra como un tratamiento para algunos tipos de cáncer. Este se utiliza de manera más común para tratar cáncer de ovario, de tejido mamario y de pulmón de célula no pequeña.

Docetaxel es un agente anti-cáncer reconocido, y es un inhibidor mitótico.

Daunorrubicina es un fármaco antineoplásico

utilizado en un número de campos terapéuticos, incluyendo como un agente anti-cáncer.

Doxorrubicina es un agente antibiótico tipo antraciclina previamente reconocido como un agente anti-tumor.

Hidroxiurea es un inhibidor de ribonucleótido reductasa antineoplásico.

Como se describe de manera más detallada en los resultados experimentales más adelante ("Experimentos B"), doxetaxel y paclitaxel son particularmente preferidos, y muestran efectos sinergistas particularmente buenos con anticuerpo anti-Hsp90. También se observa sinergismo con imatinib, doxorrubicina, daunorrubicina, e hidroxiurea cuando se combinan con anticuerpo anti-Hsp90. El agente anti-cáncer cisplatina, cuando se utiliza junto con anticuerpo anti-Hsp90 muestra resultados indiferentes. Esto confirma la naturaleza sorpresiva/inesperada del sinergismo logrado con los agentes anti-cáncer anteriores cuando se combinan con anticuerpo anti-Hsp90.

También se provee un preparado combinado que comprende:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se

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selecciona a partir del grupo que consiste de: imatinib, paclitaxel, docetaxel, daunorrubicina, doxorrubicina, e hidroxiurea,

para el uso simultáneo, por separado o en secuencia en el tratamiento de leucemia.

Los ejemplos de preparados combinados incluyen paquetes farmacéuticos que contienen el anticuerpo de (i) y por lo menos un agente anti-cáncer de (ii) en volúmenes separados (es decir no están mezclados entre sí en un preparado individual).

También se provee un método de tratamiento de leucemia que comprende administrar una cantidad terapéuticamente efectiva de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: imatinib, paclitaxel, docetaxel, daunorrubicina, doxorrubicina, e hidroxiurea,

a un paciente en necesidad de lo mismo.

La leucemia puede ser leucemia mieloide crónica o leucemia linfoide aguda, y dicho por lo menos un agente anti-cáncer puede ser imatinib.

El anticuerpo o fragmento de unión a antígeno del

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mismo puede ser específico para el epítope desplegado por un péptido que comprende la secuencia de SEQ ID NO:1.

Por ejemplo, el anticuerpo utilizado en la composición o preparado combinado puede comprender la secuencia de SEQ ID NO:2.

El agente anti-cáncer puede ser imatinib.

El inventor de la presente ha descubierto que las leucemias que se pueden tratar de manera útil incluyen leucemias que se seleccionan a partir del grupo que consiste de: leucemia mieloblástica aguda, leucemia linfoblástica aguda, leucemia mieloide crónica, y leucemia linfocítica crónica.

La leucemia puede ser leucemia mieloide crónica o leucemia linfoide aguda.

La leucemia mieloide crónica puede ser Ph1-positiva o Ph1-negativa, es decir se caracteriza por células leucémicas que contienen el cromosoma Filadelfia (Ph1-positiva), o que carecen del cromosoma Filadelfia (Ph1-negativa).

El inventor de la presente ha descubierto que las leucemias mieloides crónicas que se pueden tratar de manera útil con imatinib pueden ser ya sea Ph1-positivas o Ph1-negativas.

La leucemia puede ser leucemia mieloide crónica la cual es Ph1-positiva, y el agente anti-cáncer puede ser

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imatinib. En particular, el inventor de la presente invención ha descubierto que el tratamiento de CML que sea Ph1-positiva se puede efectuar mediante una combinación de imatinib y un anticuerpo que comprenda la secuencia de SEQ ID NO:2. Sin desear estar limitado a ninguna teoría, es posible que Hsp90 sea secuestrada por el anticuerpo que comprende la secuencia de SEQ ID NO:2, lo que a su vez significa que la tirosina cinasa Bcr/Abl anormal (la cual ocasiona la mielopoyesis desordenada encontrada en CML) está, por ejemplo, plegada de manera incorrecta, elegida como blanco para degradación de proteína, y/o se evita que ejerza sus efectos sobre las rutas mielopoyéticas.

Este tratamiento también es efectivo sobre células Ph1-positivas de CML resistentes a imatinib. Sin estar limitado a ninguna teoría, la resistencia a imatinib probablemente se deba a mutaciones coleccionadas en la tirosina cinasa Bcr/Abl anormal que puedan, por ejemplo, evitar que el fármaco se una a la proteína y/o interfiera con el modo de acción del fármaco. En células resistentes a imatinib, es posible que el secuestro de Hsp90 por parte del anticuerpo que comprende la secuencia de SEQ ID NO:2, ocasione que la tirosina cinasa anormal mutada sea, por ejemplo, plegada incorrectamente, elegida como blanco para degradación de proteína, y/o que se evite que ejerza sus efectos sobre las rutas mielopoyéticas. El secuestro de

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Hsp90, el cual normalmente sirve para "amortiguar" las mutaciones genéticas asociadas con células cancerosas mediante unión a proteínas anormales y bloquear su expresión, también puede ocasionar que se libere una variedad de mutaciones que juntas demuestran ser sintéticamente letales para la célula del tumor. Las células normales, las cuales carecen de la inestabilidad genética de las células tumorales, se ven relativamente no afectadas.

De manera adicional, y en forma particularmente sorpresiva, el inventor de la presente ha descubierto que el tratamiento de CML que sea Ph1-negativa se puede efectuar mediante una combinación de imatinib y un anticuerpo que comprenda la secuencia de SEQ ID NO:2.

La leucemia puede ser leucemia mieloide crónica que sea Ph1-negativa, y el agente anti-cáncer puede ser imatinib.

Este hallazgo es sorpresivo debido a que las células de CML Ph1-negativas carecen de la proteína tirosina cinasa anormal asociada con células Ph1-positivas. Sin embargo, y sin desear estar limitado a ninguna teoría, es posible que existan niveles bajos o basales de esta cinasa (ya sea anormales o de alguna otra manera) en células Ph1-negativas, y como se describió anteriormente, el secuestro de Hsp90 es por parte del anticuerpo que

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comprende la secuencia de SEQ ID NO:2, lo que significa que la proteína tirosina cinasa se pliegue, por ejemplo de manera incorrecta, o se elija como blanco para degradación de proteína, o en alguna otra manera se evite que ejerza sus efectos sobre las rutas mielopoyéticas. También podría ser el caso que al secuestrar a Hsp90, se liberen una variedad de mutaciones por parte de la célula de tumor las cuales juntas demuestran ser sintéticamente letales.

El efecto sorpresivo de imatinib y el anticuerpo anti-Hsp90 en células Ph1-negativas se puede deber a la presencia de la fusión TEL(ETV6)-ABL, la cual ha sido demostrada en dos casos de CML Ph1-negativa (Krystal, GW, 2004, Leukemia Research 28S1:pS53-S59), y la cual es sensible a imatinib.

La leucemia se puede caracterizar por células que sean resistentes a imatinib.

La composición o preparado de la presente invención puede comprender de manera adicional un inhibidor de Hsp90 conocido, por ejemplo GA, o 17-AAG.

Un tercer aspecto de la presente invención (Experimentos C) se refiere a medicamentos y preparados novedosos que comprenden agentes anti-cáncer efectivos junto con un anticuerpo anti-Hsp90 los cuales juntos proveen una eficacia incrementada en el tratamiento de cáncer colorectal o adenocarcinomas.

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El cáncer colorectal es un tumor maligno del colon o recto. El cáncer colorectal es una causa importante de morbilidad y mortalidad por cáncer. Es el tercer cáncer más común en hombres y el segundo cáncer más común en mujeres en el Reino Unido. El noventa y cinco por ciento de cánceres colorectales son adenocarcinomas, los cuales son cánceres de la célula glandular que reviste el interior del colon y el recto.

El tratamiento normal de cáncer colorectal generalmente es una combinación de 5-fluorouracilo y leucovorin (ácido folínico).

5-Fluorouracilo (5-FU) se utiliza para tratar una variedad de tumores sólidos, incluyendo cánceres gastrointestinales y cáncer de tejido mamario. Este se utiliza comúnmente con ácido folínico en cáncer colorectal avanzado. 5-FU se convierte en FdUMP en la célula, el cual forma un complejo con timidilato sintetasa (TS) que inhibe la síntesis de ADN, de proteína y de ARN.

El ácido folínico (leucovorin) es una vitamina que se administra en combinación con 5-FU. El ácido folínico incrementa la tasa de respuesta hacia 5-fluorouracilo, con una mejora significativa en la supervivencia libre de enfermedad y supervivencia en general. El ácido folínico incrementa el folato intracelular y estabiliza el complejo FdUMP/TS.

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Otros agentes que tienen un efecto incluyen irinotecano y oxaliplatina, la cual ha sido aprobada para uso de primera línea en pacientes con cáncer colorectal avanzado, en combinación con 5-fluorouracilo, y ácido folínico. El irinotecano o raltitrexed ha sido aprobado para uso como una monoterapia de segunda línea cuando fracasa o es inapropiada la terapia basada en fluorouracilo.

Oxaliplatina es un agente anti-cáncer reconocido y contiene un compuesto de diaminociclohexano-platino novedoso que forma entrelazamientos en el ADN y de esta manera inhibe la replicación del ADN.

"FOLFOX" es la quimioterapia de combinación utilizada comúnmente de 5-fluorouracilo, ácido folínico y oxaliplatina.

Irinotecano (CPT-11, Campto) inhibe la topoisomerasa I, una enzima de desdoblamiento de ADN esencial para la división celular, lo cual da como resultado la suspensión de replicación con rupturas en el ADN de cadena individual. En el Reino Unido, irinotecano ha sido aprobado para uso en pacientes no afectados por quimioterapia con cáncer colorectal avanzado en combinación con 5-FU/FA y como un agente individual para quimioterapia de segunda línea en pacientes que no han respondido a un régimen establecido basado en 5FU.

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Raltitrexed (ZD 1694, Tomudex) inhibe la enzima timidilato sintetasa, la cual está implicada en la síntesis de ADN. Esta es la misma enzima que es elegida como blanco por 5FU. Raltitrexed ha sido aprobada en el Reino Unido para el tratamiento paliativo de cáncer colorectal avanzado en casos en los que los regímenes basados en 5FU/FA no son tolerados o son inapropiados.

Tebbutt *et al.*, 2002, European Journal of Cancer, 38: 1000-1015; Cutsem *et al.*, 2002, Best Practice and Research Clinical Gastroenterology, 16:319-330; Beretta *et al.*, 2004, Surgical Oncology, 13:63-73; NICE guidelines for irinotecan, Oxaliplatin and raltitrexed for advanced colorectal cancer, 2002.

De conformidad con este tercer aspecto de la invención, se provee el uso de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: 5-fluorouracilo, oxaliplatina, irinotecano y raltitrexed,

en un método para fabricar un medicamento para el tratamiento de cáncer.

De manera alternativa, se provee un preparado combinado que comprende:

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(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: 5-fluorouracilo, oxaliplatina, irinotecano, y raltitrexed,

para el uso simultáneo, por separado o en secuencia en el tratamiento de cáncer.

De conformidad incluso con un aspecto adicional de esta invención se provee un método de tratamiento de cáncer que comprende administrar una cantidad terapéuticamente efectiva de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: 5-fluorouracilo, oxaliplatina, irinotecano y raltitrexed,

a un paciente en necesidad del mismo.

De preferencia, el cáncer es cáncer colorectal o adenocarcinoma.

De manera más preferida, el agente anti-cáncer 5-fluorouracilo también comprende o se administra con ácido folónico (leucovorin).

De manera adicional o como alternativa, 5-

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fluorouracilo, ácido folínico y oxaliplatina se administran juntos.

La composición o preparado de conformidad con cualquier aspecto de esta invención puede comprender de manera adicional un vehículo, diluyente o excipiente farmacéuticamente aceptable. En forma similar, cualquier método de fabricación de la presente invención o uso en la misma puede también comprender el uso de un vehículo, diluyente o excipiente farmacéuticamente aceptable. Los ejemplos de vehículos, diluyentes y excipientes farmacéuticamente aceptables son bien conocidos en la técnica, por ejemplo, véase: Remington Pharmaceutical Sciences y Farmacopea E.U.A., (1984, Mack Publishing Company, Easton, PA, E.U.A.).

Los medicamentos o preparado combinado se puede administrar, por ejemplo, por vía oral aunque esto no significa que se deban excluir otras vías de administración.

El anticuerpo o fragmento de unión a antígeno del mismo de conformidad con la presente invención se puede marcar con una marca detectable o se puede conjugar con una molécula efectora, por ejemplo un fármaco, por ejemplo un agente anti-cáncer tal como doxorrubicina, daunorrubicina, docetaxel o cisplatina, o 5-fluorouracilo, oxaliplatina, irinotecano y raltitrexed o un agente farmacéutico útil

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para tratar leucemia, por ejemplo, imatinib, paclitaxel, docetaxel, daunorrubicina, doxorrubicina, e hidroxiurea, o una toxina, tal como ricina, o una enzima, utilizando procedimientos convencionales, y la invención se extiende a dichos anticuerpos marcados o conjugados de anticuerpo.

Si se desea, se pueden utilizar mezclas de anticuerpos para diagnosis o tratamiento, por ejemplo, mezclas de dos o más anticuerpos que reconozcan epítopes diferentes de una proteína de tensión de conformidad con la invención, y/o mezclas de anticuerpos de una clase diferente, por ejemplo mezclas de anticuerpos de IgG e IgM que reconozcan el mismo epítope o epítopes diferentes de la invención.

Como se discutió anteriormente, aunque los epítopes cuaternarios desplegados por Hsp90 en complejos de chaperón múltiple han sido sugeridos como blancos apropiados para terapia, los experimentos (más adelante) muestran que de hecho un epítope lineal es un blanco útil y efectivo para terapia.

Los contenidos de cada una de las referencias discutidas en la presente invención, incluyendo las referencias citadas en las mismas, quedan incorporadas en la presente invención para referencia en su totalidad.

En casos en los que se proporcionen números de referencia "PMID:" para publicaciones, éstos son los

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números de identificación de Pub Med asignados a las mismas por la Biblioteca Nacional de Medicina de los Estados Unidos de Norteamérica, a partir de los cuales se encuentra disponible la información y resumen bibliográfico completo para cada publicación en www.ncbi.nlm.nih.gov. Este también puede proveer acceso directo a copias electrónicas de las publicaciones completas, en particular en el caso de, por ejemplo, publicaciones de PNAS, JBC y MBC.

La presente invención será evidente de manera adicional a partir de la siguiente descripción, la cual muestra, a manera de ejemplo únicamente, modalidades específicas de la composición y experimentación con la misma.

EXPERIMENTOS

Un primer conjunto de experimentos ("Experimentos A") descritos más adelante describen en forma detallada la investigación del efecto anti-cáncer de un anticuerpo anti-Hsp90 que tiene la secuencia de SEQ ID NO:2 y específico para un epítope desplegado por un péptido que tiene la secuencia de SEQ ID NO:1, utilizado por sí mismo o en combinación con los agentes anti-cáncer doxorrubicina, daunorrubicina, docetaxel, herceptina, imatinib, cisplatina, y paclitaxel.

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Un segundo conjunto de experimentos ("Experimentos B") descritos más adelante describen en forma detallada la investigación del efecto de un anticuerpo anti-Hsp90 que tiene la secuencia de SEQ ID NO:2 y específico para un epítope desplegado por un péptido que tiene la secuencia de SEQ ID NO:1, utilizado por sí solo o en combinación con los agentes anti-cáncer imatinib, paclitaxel, docetaxel, daunorrubicina, doxorrubicina, paclitaxel, cisplatina, e hidroxiurea, sobre la línea celular K562 de leucemia mielógena crónica caucásica de humano, y la línea celular KU-812 de leucemia mielógena de humano.

Un tercer conjunto de experimentos ("Experimentos C") describen más adelante con mayor detalle la investigación del efecto de un anticuerpo anti-Hsp90 que tiene la secuencia de SEQ ID NO:2 y específico para el epítope desplegado por un péptido que tiene la secuencia de SEQ ID NO:1, utilizado por sí solo o en combinación con los agentes anti-cáncer 5-fluorouracilo (5-FU) y ácido folínico (leucovorin, LV) y/u oxaliplatina sobre la línea celular HT29 de adenocarcinoma de colon de humano.

Materiales y métodos generales

A menos que se indique de otra manera, todos los procedimientos se efectúan utilizando protocolos estándar y

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siguiendo las instrucciones del fabricante en donde sea aplicable. Los protocolos estándar para diversas técnicas incluyendo PCR, clonación molecular, manipulación y determinación de secuencia, la fabricación de anticuerpos, mapeo de epítope y diseño de mimótope, cultivo celular y despliegue de fago, se describen en textos tales como McPherson, MJ *et al.* (1991, PCR: A practical approach, Oxford University Press, Oxford), Sambrook, J. y Russell, D., "Molecular Cloning: A Laboratory Manual", tercera edición, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, New York, 2001, Huynh y Davies (1985, "DNA Cloning Vol I - A Practical Approach", IRL Press, Oxford, Ed. DM Glover), Sanger, F. *et al.* (1977, PNAS USA 74(12): 5463-5467), Harlow, E. y Lane, D. ("Using Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, New York, 1998), Jung, G. y Beck-Sickinger, AG (1992, Angew. Chem. Int. Ed. Eng., 31: 367-486); Harris, M.A. y Rae, I.F. ("General Techniques of Cell Culture", 1997, Cambridge University Press, ISBN 0521 573645), "Phage Display of Peptides and Proteins: A Laboratory Manual" (Eds. Kay, BK, Winter, J., y McCafferty, J., Academic Press Inc., 1996, ISBN 0-12-402380-0).

Los reactivos y equipos útiles en, entre otros, los métodos descritos con detalle en la presente invención se pueden conseguir a partir de los similares de Amersham

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(www.amersham.co.uk), Boehringer Mannheim (www.boehringer-ingeltheim.com), Clontech (www.clontech.com), Genosys (www.genosys.com), Millipore (www.millipore.com), Novagen (www.novagen.com), Perkin Elmer (www.perkinelmer.com), Pharmacia (www.pharmacia.com), Promega (www.promega.com), Qiagen (www.qiagen.com), Sigma (www.sigma-aldrich.com) y Stratagene (www.stratagene.com).

Anticuerpo

El anticuerpo utilizado en los experimentos A y B más adelante es el que se describe en el documento WO 01/76627, y se hace referencia al mismo en la presente invención como Mycograb (RTM), que tiene la secuencia de SEQ ID NO:2 y que es específico para un epítope desplegado por el péptido que tiene la secuencia de SEQ ID NO:1. La solución básica de anticuerpo es una solución de reserva de 4 mg/ml en agua. Las diluciones adicionales se efectúan en medio completo RPMI.

Brevemente, se modifica genéticamente la secuencia de ADN de un anticuerpo previo específico para el epítope Hsp90 de *Candida albicans* descrito en los documentos GB 2240979 y EP 0406029 mediante optimización de codón para expresión en *Escherichia coli* (Operon Technologies Inc., Alameda CA, E.U.A.) y se inserta en un vector de expresión de *E. coli*. La secuencia de aminoácido

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del anticuerpo anti-Hsp90 comprende la secuencia de SEQ ID NO:2 (incluye los dominios pesado, ligero y de espaciador). El anticuerpo reconoce al epítope que comprende la secuencia de SEQ ID NO:1.

El anticuerpo anti-Hsp90 se expresa en un hospedero *Escherichia coli* y después se purifica mediante cromatografía por afinidad y una columna de intercambio de imidazol hasta 95% de pureza. Se emplean protocolos de biología molecular estándar (véase, por ejemplo Harlow & Lane, *supra*; Sambrook, J. *et al.*, 1989, Molecular Cloning: A Laboratory Manual, 2da edición, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Sambrook, J. & Russell, D., 2001, Molecular Cloning: A Laboratory Manual, 3ra edición, Cold Spring Harbor Laboratory Press, Cold Spring Harbor).

Fármacos

Cisplatina se obtiene a partir de Bristol-Myers Squibb, Mayne, suministrado como 1 mg/ml.

Docetaxel se obtiene a partir de Sigma. Inicialmente se diluyen 5 mg hasta 16 mg/ml con sulfóxido de dimetilo (DMSO).

Doxorrubicina se obtiene de Pharmacia; 5 ml suministrado como clorhidrato de doxorrubicina 2 mg/ml.

Imatinib (Glivec (RTM)), obtenido a partir de

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Novartis, se suministra como cápsulas de 100 mg. Imatinib se diluye inicialmente en agua para producir una solución de reserva de 10 mg/ml.

Pacilitaxel se obtiene a partir de Sigma, reconstituido en 250 µl de metanol y se lleva hasta 2.5 ml con agua para obtener 2 mg/ml.

Daunorrubicina se obtiene a partir de Sigma, se diluyen 5 mg en 2.5 ml de agua para obtener 2 mg/ml.

Herceptina (RTM) (Trastuzumab) se obtiene a partir de Roche y se reconstituye en 7.2 ml de agua para obtener 21 mg/ml.

Hidroxiurea se obtiene a partir de Sigma, diluyendo 1 g en 4 ml de agua para obtener 25 mg/ml.

5-Fluorouracilo (5-FU) se obtiene a partir de Sigma, se reconstituyen 96 mg en 1 ml de DMSO diluido 1/10 en medio RPMI completo para obtener 9.6 mg/ml.

Acido folínico (LV) se obtiene de Sigma; se reconstituyen 100 mg en 25 ml de agua para obtener una solución de reserva de 4 mg/ml.

Oxaliplatina se obtiene a partir de Sigma; se reconstituyen 12.5 mg en 2.5 ml de agua para obtener 5 mg/ml.

Cada uno de los fármacos anteriores se diluye adicionalmente en medio completo RPMI.

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Concentración celular y determinación de viabilidad

Las células se cuentan y se determina el porcentaje de viabilidad utilizando un hematocitómetro estándar después de teñir con un volumen igual de solución al 0.4% de azul de tripano (Sigma).

Prueba de viabilidad celular

Se determinan las viabilidades celulares después de cada experimento utilizando la prueba azul para título celular (Promega). Se retiran los medios de las células y se agregan 100 µl de medio completo nuevo seguido por 20 µl de reactivo azul para título celular. Esto se incuba a 37°C, 5% de CO_2 durante 4 horas y se lee la absorbancia a 570 nm utilizando 600 nm como una referencia. Esta prueba utiliza el colorante indicador resazurina (azul) para medir la capacidad metabólica de las células. Las células viables reducen resazurina a resofurina (rosa).

Interpretación de los datos

El crecimiento celular se evalúa como se describió anteriormente. Se determinan las concentraciones CI_{50} (la dosis de fármaco necesaria para ocasionar citotoxicidad en el 50% de las células) en forma individual para cada fármaco a través de períodos de incubación de 48

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horas.

El análisis de efecto de la mediana, una medida de sinergismo, efectos aditivos, o antagonismo basado en la ecuación de Hill, se determina empleando el método de Chou y Talalay usando el producto Calcusyn (BioSoft, Cambridge, RU - www.biosoft.com). Se calcula el CI (índice de combinación) el cual refleja sinergismo cuando es menor de 1, efectos aditivos cuando es igual a 1, y antagonismo cuando es mayor de 1, para niveles variables de efecto del fármaco. Se analizan 10 proporciones fijas de fármaco por encima y por debajo de la CI_{50} (la concentración de fármaco requerida para ejercer un efecto citotóxico de 50%) con un intervalo de 0.0156N-8N en el cual N es un valor cercano a la CI_{50} de un fármaco individual, incubando las combinaciones de fármaco con células durante 48 horas y determinando después el grado de citotoxicidad. Fa50 se define como el punto en el cual se ven afectadas el 50% de las células. Se muestran valores de CI para Fa50.

Experimentos A

Los resultados muestran que el anticuerpo produce evidencia de antagonismo con imatinib e indiferencia con paclitaxel. Existe cierto sinergismo con cisplatina y con docetaxel, pero éste último es probable a concentraciones que no se pueden lograr clínicamente. Doxorrubicina

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demuestra sinergismo a niveles de fármaco clínicamente factibles con ambas líneas celulares y de manera independiente de si existe o no un receptor de estrógeno. Los resultados logrados con doxorrubicina se clasifican como un sinergismo altamente significativo. Los resultados de daunorrubicina son igualmente impresionantes con la línea celular que tiene un receptor de estrógeno pero menos impresionantes con la línea celular negativa para receptor de estrógeno con sinergismo restringido a 6 y 12.5 mg/l. Los resultados para herceptina no muestran sinergismo contra una línea celular positiva en cuanto a receptor de estrógeno, pero se observa sinergismo con una línea celular negativa en cuanto a receptor de estrógeno.

Materiales y métodos

Línea celular e información del cultivo

La línea celular MCF7 de adenocarcinoma de tejido mamario de humano caucásico, que expresa tanto receptores de tipo silvestre como receptores variables de estrógeno así como un receptor de progesterona, se obtiene a partir de ECACC (número ECACC - 86012803).

Las otras líneas celulares utilizadas son las siguientes:

HS578T - número ECACC 86082104, carcinoma de tejido mamario de humano, epitelial. Tumorigénico en

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ratones inmuno-suprimidos y forma colonias en medio semi-sólido. Negativa en cuanto a receptor de estrógeno.

SK-BR-3 - (ATCC) adenocarcinoma de tejido mamario de humano. Positivo en cuanto a receptor de estrógeno. Sobre-expresa al gen HER2/C-erb-2.

UACC-812 - (ATCC) carcinoma de ducto, antes de la cirugía, el paciente ha sido sometido a quimioterapia extensiva. Negativa en cuanto a receptor de estrógeno, negativa en cuanto a receptor de progesterona, negativa en cuanto a glucoproteína P. Amplificación de la secuencia de oncogen HER-2/neu.

HCT116-ATCC carcinoma colorectal. Positivo para la expresión de TGF beta 1 y beta 2.

Las células se separan utilizando tripsina al 0.25%/EDTA (Sigma) y se mantienen en medio RPMI sin rojo de fenol, que contiene 10% de suero fetal de bovino, 1% de aminoácidos no esenciales, 2 mM de glutamina, 100 U/ml de penicilina, 0.1 mg/ml de estreptomicina (Sigma) a 37°C, 5% de CO_2.

Experimentos A

Efecto de Mycograb sobre células MCF7

Se separan las líneas celulares y se cuentan las células. Las células se agregan a placas de cultivo de tejidos de fondo plano de 96 cavidades o de 12 cavidades.

En el caso de las placas de 12 cavidades, se agregan 1 ml de 4 x 10^4 células/ml + 1 ml de medio. En el caso de la placa de 96 cavidades, se agregan 100 µl de una suspensión 4 x 10^4 células/ml seguido por la adición adicional de 100 µl de medio completo a la placa. Las placas se incuban durante la noche a 37°C, 5% de CO_2. Al siguiente día, se observan las células bajo microscopía de contraste de fases para asegurar que éstas se han adherido a las placas y el medio sobrenadante se elimina mediante aspiración. Después se agrega a las cavidades medio nuevo que contiene concentraciones con incrementos del doble de Mycograb (1.5-200 µg/ml) o solución reguladora para formulación o medio solo. La placa se regresa a la incubadora durante 48 horas después de lo cual se efectúa la prueba azul de título celular o se efectúan las cuentas de células viables utilizando un hematocitómetro.

Efecto de los agentes anti-cáncer doxorrubicina, daunorrubicina, herceptina, docetaxel, imatinib, paclitaxel y cisplatina sobre células MCF7

Se dividen las líneas celulares y se cuentan las células. Se agregan 100 µl de suspensión 4 x 10^4 células/ml a placas de cultivo de tejido de fondo plano de 96 cavidades, se agregan a la placa 100 µl adicionales de medio completo. Las placas se incuban después durante la

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noche a 37°C, 5% de CO_2. Al siguiente día, las células se observan bajo microscopía de contraste de fases para asegurar que éstas estén adheridas a las placas y el medio sobrenadante se elimina mediante aspiración. Se agrega medio nuevo que contiene concentraciones crecientes del fármaco de estudio (doxorrubicina 0.55-600 µg/ml, daunorrubicina 0.45-1000 µg/ml, herceptina 0.2-200 µg/ml, docetaxel 0.75-800 µg/ml, imatinib 4.5-5000 µg/ml, cisplatina 0.04-50 µg/ml, paclitaxel 1.8-1000 µg/ml) o medio solo a las cavidades. Las placas se regresan a la incubadora durante 48 horas después de lo cual se efectúan las pruebas azules de titulación de células.

Efecto de Mycograb en combinación con los agentes anti-cáncer doxorrubicina, daunorrubicina, herceptina, docetaxel, imatinib, paclitaxel y cisplatina sobre células MCF7

Se dividen las líneas celulares y se cuentan las células. Se agregan 100 µl de suspensión 4 x 10^4 células/ml a placas de cultivo de tejido de fondo plano de 96 cavidades, se agregan a la placa 100 µl adicionales de medio completo. Las placas se incuban después durante la noche a 37°C, 5% de CO_2. Al siguiente día, las células se observan bajo microscopía de contraste de fases para asegurar que éstas estén adheridas a las placas y el medio

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sobrenadante se elimina mediante aspiración. Se agregan 100 µl de medio nuevo a las placas y se establece una matriz de tipo tablero de ajedrez de Mycograb contra otro fármaco como se indica en la tabla 1 más adelante (utilizando doxorrubicina como un ejemplo) para obtener un volumen total de 200 µl por cavidad.

Las placas se regresan a la incubadora durante 48 horas después de lo cual se efectúan las pruebas azules de título celular.

También se efectúan experimentos con células HS578T utilizando las metodologías anteriores, y los resultados se proveen más adelante.

Resultados de los experimentos A

Línea celular MCF7

Cisplatina

La CI_{50} es 6 mg/l y no se presenta efecto al agregar Mycrograb excepto el sinergismo a concentraciones más altas-véase tabla 2.

Imatinib

La CI_{30} es 37.5 mg/l y existe evidencia de antagonismo con valores de CI en el intervalo de 3.3-10 con Mycograb a dosis de imatinib por debajo de 37.5 mg/l. Por

encima de esta dosis el imatinib aniquila la línea celular.

Docetaxel

La CI_{50} es 225 mg/l y existe evidencia de cierta cantidad de sinergismo con Mycograb a dosis altas de docetaxel-véase tabla 3.

Paclitaxel

La CI_{50} es 225 mg/l y se presenta indiferencia con concentraciones bajas del fármaco y sinergismo ligero a niveles altos tales como 500 mg/l de Paclitaxel. Estos niveles están fuera de aquellos que son clínicamente relevantes.

Doxorubicina

La CI_{50} es 1.75 mg/l. Se presenta sinergismo evidente con Mycograb a lo largo de un intervalo de concentraciones de fármaco-véase tabla 4.

Daunorubicina

La CI_{50} es 1 mg/l. Se presenta evidencia de sinergismo con Mycograb a lo largo de un intervalo de concentraciones de fármaco-véase tabla 5.

Herceptina

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No existe actividad detectable debido a herceptina ni tampoco evidencia de sinergismo.

Línea celular HS578T

Esta línea celular es insensible a Mycograb en concentraciones crecientes de hasta 400 mg/l. Esto no es sorpresivo en el sentido que estos tumores no son sensibles a esteroide y por lo tanto intrínsecamente improbables de responder a un inhibidor de Hsp90 tal como Mycograb. Sin embargo, en combinación con la antraciclina doxorrubicina, al igual que con daunorrubicina, y herceptina se presenta sinergismo inesperado.

Doxorrubicina

La CI_{50} es 1 mg/l. Existe evidencia de sinergismo con Mycograb a lo largo de un intervalo de concentraciones de fármaco-véase tabla 6.

Daunorrubicina

La CI_{50} es 1 mg/l. Existe cierta evidencia de sinergismo pero principalmente indiferencia con Mycograb-véase tabla 7.

Herceptina

Con HS578T, mono-herceptina no puede aniquilar el

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50% de las células en concentraciones de hasta 200 mg/l, pero en presencia de Mycograb se observa sinergismo-véase tabla 8.

Docetaxel

Este produce una CI_{50} de 50 mg/l para la línea celular HS578T y no muestra evidencia de sinergismo con Mycograb.

Cisplatina

Cisplatina tiene una CI_{50} de 12.5 mg/l para la línea celular HS578T y no muestra evidencia de sinergismo con Mycograb.

Conclusión

Existe evidencia de antagonismo con imatinib e indiferencia con Paclitaxel. Existe cierta cantidad de sinergismo con cisplatina y con docetaxel, pero el último es probable a concentraciones que no se pueden lograr clínicamente. Doxorrubicina demuestra sinergismo a niveles de fármaco clínicamente factibles con ambas líneas celulares y en forma independiente de que exista o no un receptor de estrógeno. Los resultados logrados con doxorrubicina se califican como un sinergismo altamente significativo. Los resultados de daunorrubicina son

igualmente impresionantes con la línea celular que tiene un receptor de estrógeno pero menos impresionantes con la línea celular negativa en cuanto a receptor de estrógeno con sinergismo restringido a 6 y 12.5 mg/l.

Este efecto sinergista sorpresivo anterior se observa entre el anticuerpo y algunos fármacos anti-cáncer, pero no con otros agentes anti-cáncer tales como imatinib.

Experimentos B

Un segundo conjunto de experimentos (descritos más adelante) detallan la investigación del efecto de un anticuerpo anti-Hsp90 que tiene la secuencia de SEQ ID NO:2 y específico para un epítope desplegado por un péptido que tiene la secuencia de SEQ ID NO:1, utilizado por sí solo o en combinación con los agentes anti-cáncer imatinib, paclitaxel, docetaxel, daunorrubicina, doxorrubicina, paclitaxel, cisplatina, e hidroxiurea, sobre la línea celular K562 de leucemia mielógena crónica caucásica de humano, y la línea celular KU-812 de leucemia mielógena de humano.

Los resultados muestran que el anticuerpo produce evidencia de sinergismo con imatinib, paclitaxel, docetaxel, daunorrubicina. El anticuerpo produce evidencia de cierta cantidad de sinergismo con doxorrubicina e hidroxiurea. Los resultados muestran que el anticuerpo

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produce evidencia de indiferencia con cisplatina.

Los resultados logrados con docetaxel y paclitaxel se clasifican como un sinergismo altamente significativo.

Materiales y métodos

Información de línea celular y cultivo

La línea celular KU-812 de leucemia mielógena de humano se obtiene a partir de ECACC (número ECAAC 90071807). En esta línea celular se ha detectado un cromosoma Filadelfia (Ph1). Las células son morfológicamente características de los basófilos.

La línea celular K562 de leucemia mielógena crónica caucásica de humano, se obtiene a partir de ECACC (número ECACC 89121407). K562 se establece a partir de la efusión pleural de un individuo de género femenino de 53 años de edad con leucemia mielógena crónica en crisis blástica terminal. Los estudios cariológicos sobre diversas sub-líneas de K562 la han clasificado en tres grupos (A, B, C) (Dimery, I. W. *et al.*, 1983, Exp. Hematol.; 11(7):p601-10). La línea utilizada en estos experimentos es la K562B. Los experimentos han demostrado que estas líneas son generalmente similares en términos de: morfología, cinética de crecimiento en cultivo en suspensión líquida, eficiencia de clonación en cultivo de agar blando, unión de anticuerpo

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monoclonales anti-K562, y proteínas de superficie celular. K562B ha sido comparada con K562A y K562C, con respecto a la cinética de crecimiento, marcadores de proteína de superficie celular, antígenos de superficie, citogenética y producción de hemoglobina. Se observan diferencias entre las líneas celulares, siendo la diferencia más importante que mientras que más del 90% de células K562A o C parecen ser Ph-1-positivas, menos del 15% de las células K562B contiene un Ph1 (Dimery, IW, *et al.*, 1983, Exp. Hematol.; 11(7):p601-10). Aunque las pruebas citogenéticas no revelan la presencia de un cromosoma Ph1 verdadero, parece ser que K562 contiene parte de un cromosoma Ph1 el cual está amplificado por lo menos 4 veces. Esta parte de un cromosoma Ph1 codifica para un transcrito quimérico *bcr/c-abl*, el cual cuando se traduce produce una proteína de fusión *bcr/c-abl* (Grosveld, G., *et al.*, 1986, Mol. Cell. Biol. 6, No. 2: p607-616). La proteína de fusión de *bcr/c-abl* posee actividad de tirosina cinasa activada la cual es la responsable de la patogénesis de CML.

Las células se mantienen entre 2 x 10^6 y 9 x 10^6 células/ml en medio RPMI 1640 sin rojo de fenol, que contiene 10% de suero fetal de bovino, 2 mM de glutamina, 100 U/ml de penicilina, 0.1 mg/ml de estreptomicina (Sigma) a 37°C, 5% de CO_2.

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Experimentos

Efecto de Mycograb sobre células K562

Se cuentan las líneas celulares. Las células se agregan a placas de cultivo de tejidos de fondo plano de 96 cavidades utilizando alícuotas de 100 µl que contienen 4 x 10^5 células/ml. Después se agrega a las cavidades medio nuevo que contiene ya sea concentraciones al doble de Mycograb (RTM) (1.5-200 µg/ml), o medio solo. La placa se regresa a la incubadora durante 48 horas después de lo cual se efectúa la prueba azul de título celular, o se determinan los conteos de células viables utilizando un hematocitómetro.

Efecto de los agentes anti-cáncer doxorrubicina, daunorrubicina, docetaxel, paclitaxel, imatinib, cisplatina e hidroxiurea sobre células K562

Se cuentan las líneas celulares. Se agregan 100 µl de 2 x 10^5 o 4 x 10^5 células/ml a placas de cultivo de tejidos de fondo plano de 96 cavidades. Las placas se incuban después durante la noche a 37°C, 5% de CO_2. Se agrega a las cavidades medio nuevo que contiene concentraciones crecientes del fármaco de estudio (doxorrubicina 0.55-600 µg/ml, daunorrubicina 0.07-100 µg/ml, docetaxel 0.75-800 µg/ml, paclitaxel 0.5-500 µg/ml, imatinib 4.5-5000 µg/ml, cisplatina 0.04-50 µg/ml) o medio

solo. Las placas se regresan a la incubadora durante 48 horas después de lo cual se efectúan las pruebas azules de titulación de células.

Efecto de Mycograb en combinación con los agentes anti-cáncer doxorrubicina, daunorrubicina, docetaxel, paclitaxel, imatinib, cisplatina e hidroxiurea sobre células K562

Se cuentan las líneas celulares. Se agregan 100 µl de 2 x 10^5, o 4 x 10^5 células/ml a placas de cultivo de tejidos de fondo plano de 96 cavidades. Las placas se incuban después durante la noche a 37°C, 5% de CO_2. Se agregan 100 µl de medio nuevo a las placas y se establece una matriz tipo tablero de ajedrez de Mycograb contra otro fármaco como se indica en la tabla 9 más adelante (utilizando doxorrubicina como un ejemplo) para obtener un volumen total de 200 µl por cavidad.

También se efectúan experimentos con células KU-812 utilizando las metodologías anteriores, y los resultados se proveen a continuación.

Resultados

Efecto de Mycograb (RTM) sobre células K562

Con la línea celular K562 Mycograb por sí solo a 12.5 µg/ml demuestra una reducción del 40% en viabilidad

celular.

Efecto de Mycograb (RTM) y agentes anti-cáncer sobre células K562

Imatinib

La CI_{50} es 16 µg/ml. Existe algo de evidencia de sinergismo entre imatinib y Mycograb (RTM) a un intervalo de concentraciones de fármaco (véase tabla 10).

Doxorrubicina

La CI_{50} es 1 µg/ml. Existe algo de evidencia de sinergismo entre doxorrubicina y Mycograb (RTM) a algunas concentraciones de fármaco pero principalmente indiferencia con Mycograb (RTM).

Daunorrubicina

La CI_{50} es 0.75 µg/ml. Existe algo de evidencia de sinergismo entre daunorrubicina y Mycograb (RTM) a concentraciones bajas de fármaco (véase tabla 11).

Docetaxel

La CI_{50} es 70 µg/ml. Existe clara evidencia de sinergismo entre docetaxel y Mycograb (RTM) a un intervalo de concentraciones de fármaco (véase tabla 12).

Paclitaxel

La CI_{50} es 32 µg/ml. Existe clara evidencia de sinergismo entre paclitaxel y Mycograb (RTM) a un intervalo de concentraciones de fármaco (véase tabla 13).

Cisplatina

La CI_{50} es 12.5 µg/ml. No existe evidencia de sinergismo entre cisplatina y Mycograb (RTM).

Hidroxiurea

La CI_{50} nunca se alcanza con hidroxiurea como el único agente. Sin embargo, existe algo de evidencia de sinergismo entre hidroxiurea y Mycograb (RTM) a concentraciones bajas de fármaco (véase tabla 14).

Línea celular KU-812

Efecto de Mycograb (RTM) sobre células KU-812

Con la línea de células KU-812, Mycograb por sí solo a 50 µg/ml demuestra una reducción del 40% en viabilidad celular.

Efecto de Mycograb (RTM) y agentes anti-cáncer sobre células KU-812

Imatinib

La CI_{50} es 0.12 µg/ml. Existe algo de evidencia de sinergismo entre imatinib y Mycograb (RTM) a concentraciones bajas de fármaco (véase tabla 15).

Sumario

Utilizando una línea de células K562, existe evidencia de sinergismo con imatinib, paclitaxel, y docetaxel. Existe evidencia de cierta cantidad de sinergismo con daunorrubicina, doxorrubicina e hidroxiurea. Se observa indiferencia con cisplatina.

Utilizando la línea de células KU-812, existe evidencia de cierta cantidad de sinergismo con imatinib.

Conclusiones

Los datos presentados en la presente invención claramente demuestran que el anticuerpo Mycograb (RTM) por sí solo puede reducir la viabilidad de líneas de células CML tanto Ph1-positivas como Ph1-negativas. Asimismo, existe un sinergismo sorpresivo entre los agentes anti-cáncer, incluyendo imatinib y el anticuerpo anti-Hsp90, en líneas de células CML Ph1-positivas. Los datos también demuestran que existe sinergismo entre paclitaxel, docetaxel, daunorrubicina, doxorrubicina, hidroxiurea, y el anticuerpo anti-Hsp90, en líneas de células de leucemia Ph1-positivas. Estos resultados permiten el uso de

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composiciones que comprenden agentes anti-cáncer tales como imatinib, junto con el anticuerpo anti-Hsp90 (Mycograb, RTM) para el tratamiento de CML. El sinergismo presentado por la combinación de agente anti-cáncer y el anticuerpo Mycograb (RTM) potencialmente permite ya sea dosis de tratamiento más bajas, lo cual sería bastante significativo dada la toxicidad problemática de muchos de los agentes anti-cáncer, y en particular imatinib, o tratamientos más prolongados y más efectivos a las mismas dosis, con lo cual se reducen los efectos secundarios no deseados.

Las implicaciones clínicas de la presente invención incluyen: (i) la producción de una combinación sinergista de agentes anti-cáncer por ejemplo imatinib, y anticuerpo anti-Hsp90 en el tratamiento de CML en caso que se convierta en el tratamiento de elección. Esto podría conducir posiblemente a una reducción en la mortalidad para CML; (ii) imatinib es tóxico, y el sinergismo provisto por la presente invención significa que se puede utilizar una dosis más baja de imatinib al tiempo que se mantiene la eficacia y se reduce de manera concomitante la toxicidad; y (iii) el efecto moderador de toxicidad del anticuerpo anti-Hsp90 podría permitir que se explore la eficacia clínica de dosis más altas de imatinib y que contribuya también a un resultado clínico mejorado.

EXPERIMENTOS C

Un tercer conjunto de experimentos (descritos más adelante) detallan la investigación del efecto de un anticuerpo anti-Hsp90 que tiene la secuencia SEQ ID NO: 2 y específico para el epítope desplegado por un péptido que tiene la secuencia SEQ ID NO: 1, utilizado por sí solo o en combinación con los agentes anti-cáncer 5-FU y ácido folínico y/u oxaliplatina sobre la línea celular HT29 de adenocarcinoma de colon de humano.

Los resultados muestran que el anticuerpo produce evidencia de sinergismo con 5-FU y ácido folínico y con oxaliplatina. Existe también evidencia de sinergismo con la combinación de cuatro fármacos de Mycograb/anticuerpo anti-Hsp90 con 5-FU, ácido folínico y oxaliplatina. Se encuentra que las concentraciones mayores de 75 µg/ml de 5-FU y 10.5 µg/ml de oxaliplatina son particularmente útiles.

Material y métodos

Información de la línea celular y cultivo

La línea de células HT29 de adenocarcinoma de colon grado II caucásico de humano, se obtiene a partir de ECACC (número ECACC 91072201).

Las células se separan utilizando tripsina al 0.25%/EDTA (Sigma) y se mantienen en medio de McCoy 5a que contiene 10% de suero fetal de bovino, 2 mM de glutamina,

100 U de penicilina, 0.1 mg de estreptomicina (Sigma) a 37°C, 5% de CO_2.

Las otras líneas celulares incluyen HCT116.

Experimentos

Efecto de Mycograb (RTM) sobre células HT29

Se separan las líneas celulares y se cuentan las células. Las células se agregan a placas de cultivo de tejidos de fondo plano de 96 cavidades o de 12 cavidades. En el caso de las placas de 12 cavidades, se agregan 1 ml de 4×10^4 células/ml o 4×10^5 células/ml más 1 ml de medio de McCoy 5a completo. En el caso de la placa de 96 cavidades, se agregan 100 µl de una suspensión 4×10^4 células/ml o 4×10^5 células/ml seguido por 100 µl adicionales de medio completo de McCoy 5a a cada cavidad. Las placas se incuban después durante la noche a 37°C, 5% de CO_2. Al siguiente día, se observan las células bajo microscopía de contraste de fases para asegurar que éstas se han adherido a las placas y el medio sobrenadante se elimina mediante aspiración. Después se agrega a las cavidades medio RPMI completo nuevo que contiene concentraciones con incrementos del doble de Mycograb (RTM) (1.5-200 µg/ml) o medio solo. La placa se regresa a la incubadora durante 48 horas después de lo cual se efectúa la prueba azul de título celular o se completan los conteos

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de células viables.

Efecto de los agentes anti-cáncer 5-FU y ácido folínico y oxaliplatina sobre células HT29

Se dividen las líneas celulares y se cuentan las células. Se agregan 100 µl de 4 x 10^4 células/ml o 4 x 10^5 células/ml a placas de cultivo de tejido de fondo plano de 96 cavidades, se agregan a la placa 100 µl adicionales de medio de McCoy 5a completo. Las placas se incuban después durante la noche a 37°C, 5% de CO_2. Al siguiente día, las células se observan bajo microscopía de contraste de fases para asegurar que éstas están adheridas a las placas y el medio sobrenadante se elimina mediante aspiración. Después se agrega a las cavidades 100 µl de medio RPMI completo nuevo que contiene concentraciones que se incrementan al doble del fármaco de estudio (5-FU 4.5-2400 µg/ml más 1 mg/ml de ácido folínico u oxaliplatina 1-500 µg/ml) o medio solo (medio + DMSO al 2.5% para el control de 5-FU). Las placas se regresan a la incubadora durante 48 horas después de lo cual se efectúan las pruebas azules de titulación de células.

Efecto de Mycograb (RTM) en combinación con los agentes anti-cáncer 5FU y ácido folínico u oxaliplatina sobre células HT29

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Se dividen las líneas celulares y se cuentan las células. Se agregan 100 µl de 4 x 10^4 células/ml o 4 x 10^5 células/ml a placas de cultivo de tejido de fondo plano de 96 cavidades, se agregan a la placa 100 µl adicionales de medio completo de McCoy 5a. Las placas se incuban después durante la noche a 37°C, 5% de CO_2. Al siguiente día, las células se observan bajo microscopía de contraste de fases para asegurar que éstas están adheridas a las placas y el medio sobrenadante se elimina mediante aspiración. Se agregan a las placas 100 µl de medio RPMI nuevo completo y se establece una matriz tipo tablero de ajedrez de Mycograb (RTM) contra el fármaco de estudio ((5-FU 4.5-2400 µg/ml más 1 mg/ml de ácido folínico u oxaliplatina 1-500 µg/ml) o medio solo (medio + DMSO al 2.5% para el control de 5-FU)) como se indica en la tabla 16 para obtener un volumen total de 200 µl por cavidad.

Efecto de Mycograb (RTM) en combinación con los agentes anti-cáncer 5FU y ácido folínico y oxaliplatina sobre células HT29

Se dividen las líneas celulares y se cuentan las líneas. Se agregan 100 µl de 4 x 10^4 células/ml o 4 x 10^5 células/ml a placas de cultivo de tejido de fondo plano de 96 cavidades, se agregan a la placa 100 µl adicionales de medio completo de McCoy 5a. Las placas se incuban después

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durante la noche a 37°C, 5% de CO_2. Al siguiente día, las células se observan bajo microscopía de contraste de fases para asegurar que éstas están adheridas a las placas y el medio sobrenadante se elimina mediante aspiración. Se agregan a las placas 100 µl de medio RPMI completo nuevo y se establece una matriz tipo tablero de ajedrez de Mycograb (RTM) contra el fármaco de estudio ((5-FU:ácido folínico:oxaliplatina a una relación de 3:1:0.42) o medio solo (medio + DMSO al 2.5% para el control de 5-FU)) al igual que para la matriz tipo tablero de ajedrez de 5-FU indicada en la tabla 16 para obtener un volumen total de 200 µl por cavidad.

Resultados

Efecto de Mycograb (RTM) sobre células HT29

Con la línea de células HT29, Mycograb (RTM) por sí solo a 125 µg/ml demuestra una reducción del 50% en la viabilidad celular.

Efecto de los agentes anti-cáncer 5FU u oxaliplatina solos y Mycograb (RTM) en combinación con los agentes anti-cáncer 5FU u oxaliplatina sobre células HT29

5-FU

La CI_{50} para 5-Fluorouracilo es 150 µg/ml. Existe

clara evidencia de sinergismo entre 5-FU y Mycograb (RTM) a un intervalo de concentraciones de fármaco, véase tablas 17-20.

Oxaliplatina

La CI_{50} para oxaliplatina es 16 µg/ml. Existe algo de evidencia de sinergismo entre oxaliplatina y Mycograb (RTM) a un intervalo de concentraciones de fármaco, tabla 18.

Efecto de Mycograb (RTM) en combinación con los agentes anti-cáncer 5FU y oxaliplatina sobre células HT29

La CI_{50} de LV/5FU/Ox es 25/75/10.5 µg/ml. Existe algo de evidencia de sinergismo entre 5-FU y oxaliplatina y Mycograb (RTM) a un intervalo de concentraciones de fármaco véase tablas 22-24.

Sumario

Los resultados muestran que el anticuerpo produce evidencia de sinergismo con 5-FU y ácido folínico u oxaliplatina y algo de evidencia de sinergismo con la combinación de cuatro fármacos de 5-FU y ácido folínico y oxaliplatina con concentraciones mayores de 75 µg/ml de 5-FU y mayores de 10.5 µg/ml de oxaliplatina. Existe evidencia de sinergismo con 5-FU y oxaliplatina, la tabla

25 presenta en forma resumida los valores de CI_{50} a DE_{50}, DE_{75} y DE_{90}.

Conclusiones

Los datos presentados en la presente invención demuestran que el anticuerpo Mycograb (RTM) por sí solo puede reducir la viabilidad de una línea celular de adenocarcinoma de colon. Existe sinergismo entre los agentes anti-cáncer, incluyendo 5-fluorouracilo y oxaliplatina y el anticuerpo anti-HSP90 en una línea celular de adenocarcinoma de colon. Los datos también demuestran sinergismo entre 5-fluorouracilo y oxaliplatina con el anticuerpo anti-HSP90 en una línea celular de adenocarcinoma de colon.

TABLA 1

Matriz tipo tablero de ajedrez de Mycograb (MG) (µg/ml) contra doxorrubicina (DR) (µg/ml)

	1	2	3	4	5	6	7	8	9	10	11	12
A	DR 150 MG 50	DR 75 MG 50	DR 37 MG 50	DR 18 5 MG 50	DR 9 MG 50	DR 4 5 MG 50	DR 2 3 MG 50	DR 1 12 MG 50	DR 0 55 MG 50	DR 0 27 MG 50	MG 50	Medio sin células
B	DR 150 MG 25	DR 75 MG 25	DR 37 MG 25	DR 18 5 MG 25	DR 9 MG 25	DR 4 5 MG 25	DR 2 3 MG 25	DR 1 12 MG 25	DR 0 55 MG 25	DR 0 27 MG 25	MG 25	Medio sin células
C	DR 150 MG 12 5	DR 75 MG 12 5	DR 37 MG 12 5	DR 18 5 MG 12 5	DR 9 MG 12 5	DR 4 5 MG 12 5	DR 2 3 MG 12 5	DR 1 12 MG 12 5	DR 0 55 MG 12 5	DR 0 27 MG 12 5	MG 12 5	Medio sin células
D	DR 150 MG 6 25	DR 75 MG 6 25	DR 37 MG 6 25	DR 18 5 MG 6 25	DR 9 MG 6 25	DR 4 5 MG 6 25	DR 2 3 MG 6 25	DR 1 12 MG 6 25	DR 0 55 MG 6 25	DR 0 27 MG 6 25	MG 6 25	Medio sin células
E	DR 150 MG 3	DR 75 MG 3	DR 37 MG 3	DR 18 5 MG 3	DR 9 MG 3	DR 4 5 MG 3	DR 2 3 MG 3	DR 1 12 MG 3	DR 0 55 MG 3	DR 0 27 MG 3	MG 3	Medio sin células
F	DR 150 MG 1 5	DR 75 MG 1 5	DR 37 MG 1 5	DR 18 5 MG 1 5	DR 9 MG 1 5	DR 4 5 MG 1 5	DR 2 3 MG 1 5	DR 1 12 MG 1 5	DR 0 55 MG 1 5	DR 0 27 MG 1 5	MG 1 5	Medio sin células
G	DR 150 MG 0 75	DR 75 MG 0 75	DR 37 MG 0 75	DR 18 5 MG 0 75	DR 9 MG 0 75	DR 4 5 MG 0 75	DR 2 3 MG 0 75	DR 1 12 MG 0 75	DR 0 55 MG 0 75	DR 0 27 MG 0 75	MG 0 75	Medio sin células
H	DR 150	DR 75	DR 37	DR 18 5	DR 9	DR 4 5	DR 2 3	DR 1 12	DR 0 55	DR 0 27	Medio más células	Medio sin células

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TABLA 2

Cisplatina	Mycograb	CI
12.5	25	0.012
25	50	0.024

TABLA 3

Mycograb	Docetaxel	CI
12.5	100	0.400
25	200	0.098
50	400	0.002

TABLA 4

Mycograb	Doxorrubicina	CI
0.75	2.25	0.249
1.5	4.5	0.261
3	9	0.248
6	18	0.476
12.5	37.5	0.186
25	75	0.155
50	150	0.159

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TABLA 5

Mycograb	Daunorrubicina	CI
0.75	0.75	0.189
1.5	1.5	0.257
3	3	0.512
6	6	0.676
12.5	12.5	0.469
25	25	0.082
50	50	0.176

TABLA 6

Doxorrubicina	Mycograb	CI
0.55	0.75	0.573
1.12	1.5	0.541
2.25	3	0.824
4.5	6	0.254
9	12	0.507
18.5	25	0.538
37	50	1.033

TABLA 7

Mycograb	Daunorrubicina	CI
0.75	0.75	1.153
1.5	1.5	1.300
3	3	1.557
6	6	0.763
12.5	12.5	0.367
25	25	1.014

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TABLA 8

Mycograb	Herceptina	CI
0.75	0.75	0.007
1.5	1.5	0.008
3	3	0.005
6	6	0.034
12.5	12.5	0.025
25	25	0.170

TABLA 9

Matriz tipo tablero de ajedrez de Mycograb (MG) (µg/ml) contra doxorrubicina (DR) (µg/ml)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Medio con células	DR 0 27	DR 0 55	DR 1 12	DR 2 3	DR 4 5	DR 9	DR 18 5	DR 37	DR 75	DR 150	Medio, sin células
B	MG 0 75	DR 0 27 MG 0 75	DR 0 55 MG 0 75	DR 1 12 MG 0 75	DR 2 3 MG 0 75	DR 4 5 MG 0 75	DR 9 MG 0 75	DR 18 5 MG 0 75	DR 37 MG 0 75	DR 75 MG 0 75	DR 150 MG 0 75	Medio sin células
C	MG 1 5	DR 0 27 MG 1 5	DR 0 55 MG 1 5	DR 1 12 MG 1 5	DR 2 3 MG 1 5	DR 4 5 MG 1 5	DR 9 MG 1 5	DR 18 5 MG 1 5	DR 37 MG 1 5	DR 75 MG 1 5	DR 150 MG 1 5	Medio, sin células
D	MG 3	DR 0 27 MG 3	DR 0 55 MG 3	DR 1 12 MG 3	DR 2 3 MG 3	DR 4 5 MG 3	DR 9 MG 3	DR 18 5 MG 3	DR 37 MG 3	DR 75 MG 3	DR 150 MG 3	Medio, sin células
E	MG 6 25	DR 0 27 MG 6 25	DR 0 55 MG 6 25	DR 1 12 MG 6 25	DR 2 3 MG 6 25	DR 4 5 MG 6 25	DR 9 MG 6 25	DR 18 5 MG 6 25	DR 37 MG 6 25	DR 75 MG 6 25	DR 150 MG 6 25	Medio sin células
F	MG 12 5	DR 0 27 MG 12 5	DR 0 55 MG 12 5	DR 1 12 MG 12 5	DR 2 3 MG 12 5	DR 4 5 MG 12 5	DR 9 MG 12 5	DR 18 5 MG 12 5	DR 37 MG 12 5	DR 75 MG 12 5	DR 150 MG 12 5	Medio, sin células
G	MG 25	DR 0 27 MG 25	DR 0 55 MG 25	DR 1 12 MG 25	DR 2 3 MG 25	DR 4 5 MG 25	DR 9 MG 25	DR 18 5 MG 25	DR 37 MG 25	DR 75 MG 25	DR 150 MG 25	Medio, sin células
H	MG 50	DR 0 27 MG 50	DR 0 55 MG 50	DR 1 12 MG 50	DR 2 3 MG 50	DR 4 5 MG 50	DR 9 MG 50	DR 18 5 MG 50	DR 37 MG 50	DR 75 MG 50	DR 150 MG 50	Medio, sin células

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TABLA 10

Imatinib (µg/ml)	Mycograb (µg/ml)	CI
1	0.75	0.033
2	1.5	0.069
4	3	0.114
8	6	0.218
16	12.5	0.366
32	25	0.558
64	50	0.799

TABLA 11

Daunorrubicina (µg/ml)	Mycograb (µg/ml)	CI
0.75	1.5	0.696
1.5	3	0.872

TABLA 12

Docetaxel (µg/ml)	Mycograb (µg/ml)	CI
1.5	1.5	0.001
3	3	0.008
6	6	0.707
12.5	12.5	0.051
25	25	0.013
50	50	0.003
100	100	0.004

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TABLA 13

Paclitaxel (µg/ml)	Mycograb (µg/ml)	CI
1	1.5	0.064
2	3	0.145
4	6	0.011
8	12.5	0.05
16	25	0.04
32	50	0.06
64	100	0.077

TABLA 14

Hidroxiurea (µg/ml)	Mycograb (µg/ml)	CI
0.3	0.75	0.798
0.6	0.75	0.694

TABLA 15

Imatinib (µg/ml)	Mycograb (µg/ml)	CI
1	0.375	0.131
2	1.5	0.093
4	3	0.016
8	6	0.001
16	12.5	0.002
32	25	0.327

TABLA 16

Matriz tipo tablero de ajedrez de Mycograb (RTM) (MG en µg/ml) contra 5-fluorouracilo (5FU en µg/ml)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Medio 2 5% de DMSO	5FU 4 5	5FU 9	5FU 18 5	5FU 37	5FU 75	5FU 150	5FU 300	5FU 600	5FU 1200	5FU 2400	Sólo medio sin células
B	2 5% de DMSO MG 3	5FU 4 5 MG 3	5FU 9 MG 3	5FU 18 5 MG 3	5FU 37 MG 3	5FU 75 MG 3	5FU 150 MG 3	5FU 300 MG 3	5FU 600 MG 3	5FU 1200 MG 3	5FU 2400 MG 3	Sólo medio sin células
C	2 5% de DMSO MG 6	5FU 4 5 MG 6	5FU 9 MG 6	5FU 18 5 MG 6	5FU 37 MG 6	5FU 75 MG 6	5FU 150 MG 6	5FU 300 MG 6	5FU 600 MG 6	5FU 1200 MG 6	5FU 2400 MG 6	Sólo medio sin células
D	2 5% de DMSO MG 12 5	5FU 4 5 MG 12 5	5FU 9 MG 12 5	5FU v MG 12 5	5FU 37 MG 12 5	5FU 75 MG 12 5	5FU 150 MG 12 5	5FU 300 MG 12 5	5FU 600 MG 12 5	5FU 1200 MG 12 5	5FU 2400 MG 12 5	Sólo medio sin células
E	2 5% de DMSO MG 25	5FU 4 5 MG 25	5FU 9 MG 25	5FU 18 5 MG 25	5FU 37 MG 25	5FU 75 MG 25	5FU 150 MG 25	5FU 300 MG 25	5FU 600 MG 25	5FU 1200 MG 25	5FU 2400 MG 25	Sólo medio sin células
F	2 5% de DMSO MG 50	5FU 4 5 MG 50	5FU 9 MG 50	5FU 18 5 MG 50	5FU 37 MG 50	5FU 75 MG 50	5FU 150 MG 50	5FU 300 MG 50	5FU 600 MG 50	5FU 1200 MG 50	5FU 2400 MG 50	Sólo medio sin células
G	2 5% de DMSO MG 100	5FU 4 5 MG 100	5FU 9 MG 100	5FU 18 5 MG 100	5FU 37 MG 100	5FU 75 MG 100	5FU 150 MG 100	5FU 300 MG 100	5FU 600 MG 100	5FU 1200 MG 100	5FU 2400 MG 100	Sólo medio sin células
H	2 5% de DMSO MG 200	5FU 4 5 MG 200	5FU 9 MG 200	5FU 18 5 MG 200	5FU 37 MG 200	5FU 75 MG 200	5FU 150 MG 200	5FU 300 MG 200	5FU 600 MG 200	5FU 1200 MG 200	5FU 2400 MG 200	Sólo medio sin células

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TABLA 17

5-Fluorouracilo y Mycograb a una relación de 0.37:1

5-FU (ug/ml)	Mycograb (ug/ml)	CI
4.5	12.5	0.001
9	25	0.004
18.5	50	0.008
37	100	0.049

TABLA 18

5-Fluorouracilo y Mycograb a una relación de 0.75:1

5-FU (ug/ml)	Mycograb (ug/ml)	CI
4.5	6	0.184
9	12.5	0.295

TABLA 19

5-Fluorouracilo y Mycograb a una relación de 12:1

5-FU (ug/ml)	Mycograb (ug/ml)	CI
37	3	0.557
75	6	0.228
150	12.5	0.198
300	25	0.540
600	50	0.250
1200	100	0.478
2400	100	0.212

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TABLA 20

5-Fluorouracilo y Mycograb a una relación de 50:1

5-FU (ug/ml)	Mycograb (ug/ml)	CI
75	1.5	0.004
150	3	0.342
300	6	0.482

TABLA 21

Oxaliplatina y Mycograb a una relación de 1.25:1

Oxaliplatina (ug/ml)	Mycograb (ug/ml)	CI
7.5	6	0.378
15.5	12.5	0.182
31	25	0.153
62.5	50	0.046
125	100	0.616
250	200	0.185

TABLA 22

5-Fluorouracilo, oxaliplatina y Mycograb a una relación de 3:1:0.42

5-FU (ug/ml)	Mycograb (ug/ml)	Oxaliplatina (ug/ml)	CI
9	3	1.3	0.053
18.5	6	2.6	0.109
37	12.5	5.25	0.239
75	25	10.5	3.120

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TABLA 23

5-Fluorouracilo, oxaliplatina y Mycograb a una relación de 3:2:0.42

5-FU (ug/ml)	Mycograb (ug/ml)	Oxaliplatina (ug/ml)	CI
9	6	1.3	0.074
18.5	12.5	2.6	0.152
37	25	5.25	0.405
75	50	10.5	1.519

TABLA 24

5-Fluorouracilo, oxaliplatina y Mycograb a una relación de 3:0.5:0.42

5-FU (ug/ml)	Mycograb (ug/ml)	Oxaliplatina (ug/ml)	CI
9	1.5	1.3	0.043
18.5	3	2.6	0.089
37	6	5.25	0.184
75	12.5	10.5	2.202

TABLA 25

Fármaco (relación)	Valores de índice de combinación a				
	DE_{50}	DE_{75}	DE_{90}	Dm	r
5-FU	N/A	N/A	N/A	297.1493	0.95571
Mycograb	N/A	N/A	N/A	96.46543	0.87307
Oxaliplatina	N/A	N/A	N/A	27.77133	0.94124
5-FU/mycograb/oxaliplatina (3:1:0.42)	0.77008	0.11419	0.02256	64.92005	0.87996
5-FU/mycograb/oxaliplatina (1.5:1:0.21)	0.85085	0.16415	0.03924	55.54798	0.97163
5-FU/mycograb/oxaliplatina (6:1:0.85)	0.62267	0.09916	0.02224	61.08042	0.9142

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NOVEDAD DE LA INVENCION

Habiendo descrito el presente invento se considera como novedad y por lo tanto se reclama como propiedad lo contenido en las siguientes:

REIVINDICACIONES

1.- El uso de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: doxorrubicina, daunorrubicina, epirrubicina, herceptina, docetaxel, y cisplatina,

en un método de fabricación de un medicamento para el tratamiento de cáncer.

2.- Un preparado combinado que comprende:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: doxorrubicina, daunorrubicina, epirrubicina, herceptina,

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docetaxel, y cisplatina,

para el uso simultáneo, por separado o en secuencia en el tratamiento de cáncer

3.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 1 ó 2, caracterizado porque dicho anticuerpo o fragmento de unión a antígeno del mismo es específico para el epítope desplegado por el péptido que tiene la secuencia de SEQ ID NO:1.

4.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 1-3, caracterizado porque dicho anticuerpo comprende la secuencia de SEQ ID NO:2.

5.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 1-4, caracterizado porque dicho cáncer se selecciona a partir del grupo que consiste de: fibrosarcoma, cáncer de tejido mamario, de próstata, melanoma, leucemia, linfomas, cáncer de colon, de célula germinal testicular, de páncreas, ovárico, del endometrio, de tiroides, y de pulmón.

6.- Un método de tratamiento de cáncer que comprende administrar una cantidad terapéuticamente efectiva de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: doxorrubicina, daunorrubicina, epirrubicina, herceptina, docetaxel, y cisplatina,

a un paciente en necesidad del mismo.

7.- El uso de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90;

en un método de fabricación de un medicamento para el tratamiento de leucemia.

8.- El uso de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: imatinib, paclitaxel, docetaxel, daunorrubicina, doxorrubicina, e hidroxiurea,

en un método de fabricación de un medicamento para el tratamiento de leucemia.

9.- Una preparado combinado que comprende:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: imatinib, paclitaxel, docetaxel, daunorrubicina, doxorrubicina, e hidroxiurea,

para uso simultáneo, por separado o en secuencia en el tratamiento de leucemia

10.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 7-9, caracterizado porque dicho anticuerpo o fragmento de unión a antígeno del mismo es específico para el epítope desplegado por el péptido que tiene la secuencia de SEQ ID NO:1.

11.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 7-10, caracterizado porque dicho anticuerpo comprende la secuencia de SEQ ID NO:2.

12.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 7-11, caracterizado porque dicha leucemia se selecciona a partir del grupo que consiste de: leucemia mieloblástica aguda, leucemia linfoblástica aguda, leucemia mieloide crónica, y leucemia linfocítica crónica.

13.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 7-12, caracterizado porque dicho por lo menos un agente anti-cáncer es imatinib.

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14.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 7-13, caracterizado porque dicha leucemia es leucemia mieloide crónica o leucemia linfoide aguda.

15.- El uso o preparado combinado de conformidad con la reivindicación 14, caracterizado porque dicha leucemia se caracteriza por células que son positivas para el cromosoma Filadelfia, o células que son negativas para el cromosoma Filadelfia.

16.- El uso o preparado combinado de conformidad con la reivindicación 14, caracterizado porque dicho agente anti-cáncer es imatinib, y dicha leucemia se caracteriza por células que son positivas para el cromosoma Filadelfia.

17.- El uso o preparado combinado de conformidad con la reivindicación 14, caracterizado porque dicho agente anti-cáncer es imatinib, y dicha leucemia se caracteriza por células que son negativas para el cromosoma Filadelfia.

18.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 7-17, caracterizado porque dicha leucemia se caracteriza por células que son resistentes a imatinib.

19.- Un método de tratamiento de leucemia que comprende administrar una cantidad terapéuticamente efectiva de:

(i) un anticuerpo o un fragmento de unión a

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antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: imatinib, paclitaxel, docetaxel, daunorrubicina, doxorrubicina, e hidroxiurea,

a un paciente en necesidad del mismo.

20.- El método de conformidad con la reivindicación 19, caracterizado porque dicha leucemia es leucemia mieloide crónica, y dicho por lo menos un agente anti-cáncer es imatinib.

21.- El uso de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: 5-fluorouracilo, oxaliplatina, irinotecano, y raltitrexed,

en un método de fabricación de un medicamento para el tratamiento de cáncer.

22.- Una preparado combinado que comprende:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se

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selecciona a partir del grupo que consiste de: 5-fluorouracilo, oxaliplatina, irinotecano, y raltitrexed, para el uso simultáneo, por separado o en secuencia en el tratamiento de cáncer

23.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 21 ó 22, caracterizado porque dicho anticuerpo o fragmento de unión a antigeno del mismo es específico para el epítope desplegado por el péptido que tiene la secuencia de SEQ ID NO:1.

24.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 21-23, caracterizado porque dicho anticuerpo comprende la secuencia SEQ ID NO:2.

25.- El uso o preparado combinado de conformidad con cualquiera de las reivindicaciones 21-24, caracterizado porque dicho cáncer se selecciona a partir del grupo que consiste de: fibrosarcoma, adenocarcinoma, cáncer de tejido mamario, de próstata, melanoma, leucemia, linfomas, cáncer de colon, colorectal, cáncer de célula germinal testicular, pacreático, ovárico, del endometrio, de la tiroides, y de pulmón.

26.- El uso o preparado combinado de conformidad con la reivindicación 25, caracterizado porque dicho cáncer es cáncer colorectal o adenocarcinoma.

27.- Un método de tratamiento de cáncer que

comprende administrar una cantidad terapéuticamente efectiva de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: 5-fluorouracilo, oxaliplatina, irinotecano, y raltitrexed,

a un paciente en necesidad del mismo.

28.- El uso, preparado combinado o método de conformidad con cualquiera de las reivindicaciones 21 a 27, caracterizado porque el agente anti-cáncer es 5-fluorouracilo y comprende también ácido folinico (leucovorin).

29.- El uso, preparado combinado o método de conformidad con la reivindicación 28, caracterizado porque el agente anti-cáncer comprende 5-fluorouracilo, ácido folínico (leucovorin) y oxaliplatina.

30.- El método de conformidad con cualquiera de las reivindicaciones 6, 19, 20, 27, 28 ó 29, caracterizado porque dicha composición o preparado combinado se administra por vía oral.

31.- El uso, preparado combinado o método de conformidad con cualquiera de las reivindicaciones 1-30, caracterizado porque dicho anticuerpo o fragmento de unión

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a antígeno está marcado con una marca detectable.

32.- El uso, preparado combinado o método de conformidad con cualquiera de las reivindicaciones 1-31, caracterizado porque dicho anticuerpo o fragmento de unión a antígeno está conjugado con una molécula efectora.

33.- La invención como se describió sustancialmente en la presente invención con referencia a los ejemplos.

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RESUMEN DE LA INVENCION

La presente invención se refiere a medicamentos y preparados novedosos que comprende agentes anti-cáncer efectivos junto con un anticuerpo anti-Hsp90 los cuales juntos proveen una eficacia incrementada en el tratamiento de cáncer, y leucemia.

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LISTADO DE SECUENCIAS

<110> NeuTec Pharma plc
Burnie, James P
Matthews, Ruth C
Carter, Tracey

<120> Tratamiento de Cáncer

<130> WA/M101423WO

<150> US 60/654,458
<151> 2005-02-22

<150> US 60/614,423
<151> 2004-09-30

<150> GB 0503566.2
<151> 2005-02-21

<150> GB 0414885.4
<151> 2004-07-02

<150> GB 0420845.0
<151> 2004-09-20

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<223> Sintética

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Glu Trp Met Gly Lys Ile Asp Pro Gly Asp Ser Tyr Ile Asn Tyr Ser
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Pro Ser Phe Gln Gly His Val Thr Ile Ser Ala Asp Lys Ser Ile Asn
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Tyr Tyr Cys Ala Arg Gly Gly Arg Asp Phe Gly Asp Ser Phe Asp Tyr
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Ala Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Thr Leu
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Tyr Cys Gln His Leu Asn Ser Tyr Pro Leu Thr Phe Gly Gly Gly Thr
225 230 235 240

Lys Val Asp Ile Lys Arg Ala Ala
245

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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To:

ARENDS, William, Gerrit
Lloyd Wise, McNeight & Lawrence
Commonwealth House
1-19 New Oxford Street
London WC1A 1LW
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	05.07.2006

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
WAMP 101423-WO	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2005/002545	30.06.2005	02.07.2004

Applicant
NEUTEC PHARMA PLC et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel +49 89 2399 - 0 Tx: 523656 epmu d
Fax +49 89 2399 - 4465

Authorized Officer

Cleere, PARTNER | WA | FILE NO.
Tel. +49 89 2399-7713 | | mp101
| | GP | 423 WO

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
WAMP 101423-WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2005/002545	30.06.2005	02.07.2004

International Patent Classification (IPC) or national classification and IPC

INV. A61K39/395 C07K16/18

Applicant

NEUTEC PHARMA PLC et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10.04.2006	05.07.2006

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

Hermann, P

Telephone No. +49 89 2399-7109

Form PCT/IPEA/409 (Cover Sheet) (April 2005)

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Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-44 as originally filed

   Sequence listings part of the description, Pages

   1, 2 as originally filed

   Claims, Numbers

   1-33 as orginally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 6, 19, 20, 27-32 (for I.A.) 33 (in full)

because:

☒ the said international application, or the said claims Nos. 6, 19, 20, 27-32 (for I.A.) relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*.

☒ no international search report has been established for the said claims Nos. 33 (in full)

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-32
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	3, 10
	No:	Claims	1, 2, 4-9, 11-32
Industrial applicability (IA)	Yes:	Claims	1-5, 7-18, 21-26
	No:	Claims	-

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 6, 19, 20, 27-32 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Subject-matter for which no international search report has been established have not been examined (cf Rule 66.1 PCT).
Therefore, no opinion is provided with respect to the provisions of Art.33(1) PCT (i.e. novelty, inventive step and industrial applicability) for claim 33.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. **Reference is made to the following documents:**

   D1: Maloney A. *et al.* - "HSP90 as a new therapeutic target for cancer therapy: the story unfolds." - 2002 - *Expert Opinion on Biological Therapy*, **2**: 3-24
   D2: Schwartz J. *et al.* - "Novel targeted and immunotherapeutic strategies in chronic myeloid leukemia." - 2003 - *Seminars in Hematology*, **40**: 87-96
   D3: Banerji U. *et al.* - "The clinical applications of heat shock protein inhibitors in cancer - present and future." - 2003 - *Current Cancer Drug Targets*, **3**: 385-390
   D4: WO-A-01/76627 (Neutec Pharma Plc; Burnie J. P.) 18 October 2001

2. None of the documents at hand during preliminary examination discloses the subject-matter of present claims 1-32 said claims therefore fulfill the requirements of Article 33(2) PCT.

3. However claims 1, 2, 4-9, 11-32 do not appear to involve an inventive step contrary to

the requirements of Article 33(3) PCT the reasons being as follows:

As reported in D1, D2 or D3, Heat Shock Protein 90 (HSP90) is a very well known target for the development of new drugs for cancer therapy. Moreover D1, D2 and D3 reports that the new therapy should be designed to inhibit the interaction of HSP90 with its well known ligands (cf. D1 p. 8 left-hand column 1st § - p. 9 left-hand column 1st §, p. 17 left-hand column 6th § - p. 19 right-hand column 3rd §; D2 p. 93 left-hand column 2nd §; D3 p. 388 left-hand column 7th § - p. 389 right-hand column 3rd §), and all three documents taken separately already disclosed the synergism or benefic combination of HSP90 known inhibitors such as geldanamycin or its derivative 17-allylamino, 17-demethoxygeldanamycin (17AAG) with other well known anti-cancer drugs such as paclitaxel, doxorubicin (cf. D1 p. 16 left-hand column 3rd §; D2 p. 93 left-hand column 2nd §) or Cisplatin (cf. D3 p. 387 right-hand column 4th §).

Therefore in view of those closest prior-art D1, D2 or D3 taken separately the problem to be solved by present independent claims 1, 2, 6-9, 19, 21, 22 and 27 can be considered as the provision of a new HSP90 inhibitor to be used alone or in combination with conventional anticancer drugs for the treatment of cancer.

The solution to said technical problem, i.e. the use of a monoclonal antibody directed against HSP90 is not considered inventive because, for the skilled person, it is considered to be routine in the art to use a specific monoclonal antibody to inhibit a receptor/ligand interaction. Therefore the examining division consider that the use of anti-HSP90 monoclonal antibody as alternative to the known HSP90 inhibitors constitutes an obvious option the skilled person would select without the exercise of inventive skills. Furthermore the successful use of monoclonal antibodies directed to specific proteins in anti-cancer therapy, has already been reported (see for example D1 p. 17 right-hand column 3rd §) and would therefore motivate the skilled person to directly test, in an anti-cancer assay, already known anti-HSP90 monoclonal antibodies such as that disclosed in D4, or to develop further monoclonal antibodies directed to specific epitopes on HSP90 and would therefore arrive to the solution of the claimed invention and claims 1, 2, 6-9, 19, 21, 22 and 27 lack inventive step contrary to the requirem nts of Article 33(3) PCT.

4. In the light of the arguments presented by the applicant in his letter of reply dated April 7th, 2006, it appears that an inventive step for the present application could only be acknowledged if the antibody to which the claimed subject-matter relate would be characterized by the fact that it is specific for the HSP-90 epitope having the sequ nce of SEQ ID NO: 1 (i.e. the subject-matter of dependent claim 3 or claim 10; Articles 33(1) and (3) PCT).
Indeed it would not have been obvious for the person skilled in the art and having knowledge of the prior art documents cited in the International Search Report, to raise and select monoclonal antibodies specific for the said epitope within HSP-90, and obtain antibodies able to display the synergistic activity observed against cancer when used in therapy in combination with other well known anti-cancer drugs. It should be noted that the data given in the application are all resulting from experiments made by using Mycograb antibody in combination with well known anti-cancer agents. Said mycograb antibody is specific to the epitope cited above, and, in the absence of evidence to the contrary, it is unlikely that other antibodies specific for other epitopes within HSP-90 would provide identical surprising effect.

For similar reasons, in inventive step for the subject-matter of dependent claims 4-5, 11-18, 20, 23-26 and 28-32 could also be acknowledged, if the antibody of claims 1, 2, 6-9, 19, 21, 22, 27 to which they refer, would be characterized by the fact that it is specifically recognising the epitope defined by SEQ ID 1.
However, since at present the subject-matter of claims 4-5, 10-18, 20, 23-26 and 28-32 encompasses any antibody recognising HSP-90 or antigen binding fragment thereof, and given the fact that in the absence of evidence to the contrary, it is considered unlikely that an antibody recognising another epitope of HSP-90 than that defined in claim 3 or 10, would provide similar effects as those obtained with the use of mycograb antibody, an inventive step for claims 4-5, 10-18, 20, 23-26 and 28-32 cannot be acknowledged, contrary to the requirements of Article 33(1) and (3) PCT.

5. **Industrial applicability (Article 33(4) PCT)**

For the assessment of the present claims 6, 19, 20, 27-32 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for

example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

PATENT COOPERATION TREATY

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From the INTERNATIONAL SEARCHING AUTHORITY

To:
LLOYD WISE, MCNEIGHT & LAWRENCE
Commonwealth House
Attn. Arends, William Gerrit
1-19 New Oxford Street
London
WC1A 1LW
UNITED KINGDOM

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference WA/MP101423-WO	Date of mailing (day/month/year) 21/09/2005
International application No. PCT/GB2005/002545	FOR FURTHER ACTION See paragraphs 1 and 4 below
Applicant NEUTEC PHARMA PLC	International filing date (day/month/year) 30/06/2005

1. [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes, 1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 740.14.35

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau, if the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until **30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

WA

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax. (+31-70) 340-3016	Authorized officer Catriona Cleere

MP101 423WO

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NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1)

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b))

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

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The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added " or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence)

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
WA/MP101423-WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB2005/002545	30/06/2005	20/09/2004

Applicant

NEUTEC PHARMA PLC

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1 **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [X] **Certain claims were found unsearchable** (See Box II).

3. [ ] **Unity of invention is lacking** (see Box III).

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5 With regard to the **abstract**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings**,

a the figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] as selected by this Authority, because the applicant failed to suggest a figure.

[ ] as selected by this Authority, because this figure better characterizes the invention.

b. [X] none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2005/002545

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   - [X] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material

   - [X] in written format
   - [X] in computer readable form

   c. time of filing/furnishing

   - [X] contained in the international application as filed
   - [X] filed together with the international application in computer readable form
   - [ ] furnished subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2005/002545

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/395 C07K16/18

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)
IPC 7 C07K 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, WPI Data, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	MALONEY ALISON ET AL: "HSP90 as a new therapeutic target for cancer therapy: the story unfolds." EXPERT OPINION ON BIOLOGICAL THERAPY. JAN 2002, vol. 2, no. 1, January 2002 (2002-01), pages 3-24, XP009053600 ISSN: 1471-2598 the whole document	1-33

-/--

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

* Special categories of cited documents

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

"E" earlier 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)

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 September 2005	21/09/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040 Tx 31 651 epo nl, Fax (+31-70) 340-3016	Hermann, P

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Form PCT/ISA/210 (second sheet) (January 2004)

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHWARTZ JOSEPH ET AL: "Novel targeted and immunotherapeutic strategies in chronic myeloid leukemia." SEMINARS IN HEMATOLOGY. JAN 2003, vol. 40, no. 1, January 2003 (2003-01), pages 87-96, XP009053575 ISSN: 0037-1963 cited in the application the whole document	1-33
X	BANERJI UDAI ET AL: "The clinical applications of heat shock protein inhibitors in cancer - present and future." CURRENT CANCER DRUG TARGETS. OCT 2003, vol. 3, no. 5, October 2003 (2003-10), pages 385-390, XP009053559 ISSN: 1568-0096 cited in the application the whole document	1-33
X	WO 00/61578 A (SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH; ROSEN, NEAL; KUDUK, SCO) 19 October 2000 (2000-10-19) the whole document	1-33
A	WO 01/76627 A (NEUTEC PHARMA PLC; BURNIE, JAMES, PETER) 18 October 2001 (2001-10-18) the whole document	1-33
A	WHITESELL L ET AL: "Inhibition of heat shock protein HSP90-pp60-v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 91, no. 18, 16 August 1994 (1994-08-16), pages 8324-8328, XP002175506 ISSN: 0027-8424 the whole document	1-33

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Claims Nos.: 33

Although claims 6, 19, 20, 27-32 are directed to a method of treatment of the human/animal body by therapy (Rule 39.1(iv) PCT), the search has been carried out and based on the alleged effects of the compound/composition.

Claim 33 does not contain any characteristic features defining the subject-matter for which protection is sought, therefore claim 33 lacks clarity (Article 6 PCT) to such an extent that no meaningful search could be performed as regards its subject-matter.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2005/002545

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Box 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. [X] Claims Nos.: 33
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

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).

Box 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 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.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB2005/002545

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Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0061578	A	19-10-2000	AU	769235 B2	22-01-2004
			AU	4223500 A	14-11-2000
			CA	2370007 A1	19-10-2000
			EP	1169319 A1	09-01-2002
			JP	2002541255 T	03-12-2002
WO 0176627	A	18-10-2001	AU	4089001 A	23-10-2001
			BR	0109846 A	03-06-2003
			CA	2401836 A1	18-10-2001
			CN	1420786 A	28-05-2003
			EP	1267925 A1	02-01-2003
			JP	2003530357 T	14-10-2003
			NO	20024815 A	02-12-2002
			NZ	520899 A	24-03-2005
			PL	358394 A1	09-08-2004
			US	2003180285 A1	25-09-2003

PATENT COOPERATION TREATY

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From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2005/002545	30.06.2005	30.09.2004

International Patent Classification (IPC) or both national classification and IPC
A61K39/395, C07K16/18

Applicant
NEUTEC PHARMA PLC

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx. 523656 epmu d
Fax +49 89 2399 - 4465

Authorized Officer

Hermann, P

Telephone No. +49 89 2399-7109

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2005/002545

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3 ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2005/002545

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 6, 19, 20, 27-32 (for I.A.) 33 (in full)

because:

☒ the said international application, or the said claims Nos. 6, 19, 20, 27-32 (for I.A.) relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos 33 (in full)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

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Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-32
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-32
Industrial applicability (IA)	Yes:	Claims	1-5, 7-18, 21-26
	No:	Claims	-

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 6, 19, 20, 27-32 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Subject-matter for which no international search report has been established have not been examined (cf Rule 66.1 PCT).
Therefore, no opinion is provided with respect to the provisions of Art.33(1) PCT (i.e. novelty, inventive step and industrial applicability) for claim 33.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. **Reference is made to the following documents:**

   D1: Maloney A. *et al.* - "HSP90 as a new therapeutic target for cancer therapy: the story unfolds." - 2002 - *Expert Opinion on Biological Therapy*, 2: 3-24
   D2: Schwartz J. *et al.* - "Novel targeted and immunotherapeutic strategies in chronic myeloid leukemia." - 2003 - *Seminars in Hematology*, 40: 87-96
   D3: Banerji U. *et al.* - "The clinical applications of heat shock protein inhibitors in cancer - present and future." - 2003 - *Current Cancer Drug Targets*, 3: 385-390
   D4: WO-A-01/76627 (Neutec Pharma Plc; Burnie J. P.) 18 October 2001

2. None of the documents at hand during preliminary examination discloses the subject-matter of present claims 1-32 said claims therefore fulfil the requirements of Article 33(2) PCT.

3. However claims 1-32 do not appear to involve an inventive step contrary to the

requirements of Article 33(3) PCT the reasons being as follows:

As reported in D1, D2 or D3, Heat Shock Protein 90 (HSP90) is a very well known target for the development of new drugs for cancer therapy. Moreover D1, D2 and D3 reports that the new therapy should be designed to inhibit the interaction of HSP90 with its well known ligands (cf. D1 p. 8 left-hand column 1st § - p. 9 left-hand column 1st §, p. 17 left-hand column 6th § - p. 19 right-hand column 3rd §; D2 p. 93 left-hand column 2nd §; D3 p. 388 left-hand column 7th § - p. 389 right-hand column 3rd §), and all three documents taken separately already disclosed the synergism or benefic combination of HSP90 known inhibitors such as geldanamycin or its derivative 17-allylamino, 17-demethoxygeldanamycin (17AAG) with other well known anti-cancer drugs such as paclitaxel, doxorubicin (cf. D1 p. 16 left-hand column 3rd §; D2 p. 93 left-hand column 2nd §) or Cisplatin (cf. D3 p. 387 right-hand column 4th §).

Therefore in view of those closest prior-art D1, D2 or D3 taken separately the problem to be solved by present independent claims 1, 2, 6-9, 19, 21, 22 and 27 can be considered as the provision of a new HSP90 inhibitor to be used alone or in combination with conventional anticancer drugs for the treatment of cancer.

The solution to said technical problem, i.e. the use of a monoclonal antibody directed against HSP90 is not considered inventive because, for the skilled person, it is considered to be routine in the art to use a specific monoclonal antibody to inhibit a receptor/ligand interaction. Therefore the examining division consider that the use of anti-HSP90 monoclonal antibody as alternative to the known HSP90 inhibitors constitutes an obvious option the skilled person would select without the exercise of inventive skills. Furthermore the successful use of monoclonal antibodies directed to specific proteins in anti-cancer therapy, has already been reported (see for example D1 p. 17 right-hand column 3rd §) and would therefore motivate the skilled person to directly test, in an anti-cancer assay, already known anti-HSP90 monoclonal antibodies such as that disclosed in D4, or to develop further monoclonal antibodies directed to specific epitopes on HSP90 and would therefore arrive to the solution of the claimed invention and claims 1, 2, 6-9, 19, 21, 22 and 27 lack inventive step contrary to the requirements of Article 33(3) PCT.

4. In view of the prior-art documents at hand, dependent claims 3-5, 10-18, 20, 23-26 and 28-32 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with respect to inventive step (Article 33(3) PCT) since these additional features appear to be conventional and do not appear to result in any unexpected effect (Article 33(3) PCT).

5. **Industrial applicability (Article 33(4) PCT)**

For the assessment of the present claims 6, 19, 20, 27-32 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

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PETITOR

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Industria y Comercio
SUPERINTENDENCIA

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL

1) SOLICITUD DE:

☒ Patente de invención ☐ Patente de Modelo de Utilidad

☐ Capitulo I. ☒ Capitulo II.

2) SOLICITANTE (71)	**Nombre: NEUTEC PHARMA PLC** **Dirección: Central Manchester & Mancheter, Children´s University Holspitals NHS Trust, Oxford Road, Manchester M13 9WL, Gran Bretaña** **Nacionalidad o domicilio: Manchester, Gran Bretaña** **Lugar de constitución: Gran Bretaña**	**IDENTIFICACIÓN** **C.C.** ☐ **NIT:** ☐ **C.E.** ☐ **OTRO** ☐ **Cual** _____ **NÚMERO** _____
	Teléfono: Fax: **E. Mail:**	
3) REPRESENTANTE O APODERADO	Nombre: DILIA MARÍA RODRÍGUEZ D´ALEMAN Dirección: Carrera 11 N°. 86-53 Piso 6 Bogotá D.C. Teléfono: 6181088 Fax: 6350824 E. Mail: info@clarkemodet.com.co	**IDENTIFICACIÓN** **C.C.** ☒ **NIT:** ☐ **C.E.** ☐ **OTRO** ☐ **NÚMERO:** 41.654.882 **TP:** 20824 CSJ
4) INVENTOR (ES) (72)	**Nombre: 1. BURNIE, James, Peter; 2. MATTHEWS, Ruth, Christine; 3. CARTER, Tracey** **Dirección: 1, 2 y 3 Neutec Pharma PLC, Central Manchester & Manchester, Childresn´s University Hospitals, NHS Trust, Oxford Road, Manchester M13 9 WL, Gran Bretaña** **Nacionalidad o Domicilio: Todos los anteriores, Gran Bretaña**	

5) PCT (86)

Solicitud Internacional No. PCT/GB2005/002545 Fecha: Junio 30 de 2005

Publicación Internacional No. WO 2006/003384 A1 Fecha: Enero 12, 2006

6)Título (54) TRATAMIENTO DE CÁNCER

7) Clasificación Internacional: A61K 39/395, C07K 16/18

8) PRIORIDAD

SI ☒ **NO** ☐

33) País de Origen	(32) Fecha	(31) Número de Solicitud
Gran Bretaña	Julio 2, 2004	0414885.4
Gran Bretaña	Sept. 20, 2004	0420845.0
Estados Unidos	Sept. 30, 2004	60/614,423
Gran Bretaña	Febrero 21, 2005	0503566.2
Estados Unidos	Feb. 22, 2005	60/654.458

9) Comprobante de pago N°.

Instrucciones para el diligenciamiento del presente formulario. Ver reverso de esta pagina

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NOVEDAD DE LA INVENCION

Habiendo descrito el presente invento se considera como novedad y por lo tanto se reclama como propiedad lo contenido en las siguientes:

REIVINDICACIONES

1.- El uso de:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: doxorrubicina, daunorrubicina, epirrubicina, herceptina, docetaxel, y cisplatina,

en un método de fabricación de un medicamento para el tratamiento de cáncer.

2.- Un preparado combinado que comprende:

(i) un anticuerpo o un fragmento de unión a antígeno del mismo específico para por lo menos un epítope de Hsp90; y

(ii) por lo menos un agente anti-cáncer que se selecciona a partir del grupo que consiste de: doxorrubicina, daunorrubicina, epirrubicina, herceptina,

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
Radi: 07-005775- -00000-0000
Hoc: 2004-01-30 10:38:37 Usp. 2000 NUEVOSRADICADOS
Ins. 11 PATENTES
Evi: 370 PAGINA NACIONAL
Act 411 PRESENTACION Folios: 1/0

Industria y Comercio
SUPERINTENDENCIA

REQUISITOS MINIMOS PARA ADMISION A TRAMITE
PCT

PATENTE DE INVENCION [✓] MODELO DE UTILIDAD [ ]

- Indicación de que se solicita la concesión de una patente [✓]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [✓]
- Descripción de la invención [✓]
- Dibujos de ser estos pertinentes [ ]
- Comprobante de pago de las tasas establecidas [✓]

COMPLETA [✓] INCOMPLETA [ ]

DISEÑO INDUSTRIAL [ ]

- Indicación de que se solicita el registro de Diseño Industrial [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

ESQUEMA DE TRAZADO [ ]

- Indicación de que se solicita el registro de un Esquema de Trazado [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica del esquema trazado [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

Firma Responsable

Fecha_____

Carrera 13 No. 27-00 Piso 5, 7 y 10
Conmutador: 3 82 08 40
Fax: 350 52 20
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia

REPUBLICA DE COLOMBIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCI

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Bogotá,
2020

Doctor(a)
RODRIGUEZ DILIA MARIA

REFERENCIA	Radicación No.	7 8776
	Solicitud internacional	PCT/GB2005/002545
	Fecha inicio fase nacional	02/02/2007
	Trámite	11
	Evento	379
	Actuación	430
	Oficio No.	2586

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486.

2. La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha_____.
lerazo 23 FEB. 2007