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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E. S. D.

REF: Expediente 07-023-376

TÍTULO SOLICITADO: MÉTODOS DE UTILIZAR LIGANDOS DE RECEPTOR DE MUERTE CELULAR Y ANTICUERPOS CD20.

PUBLICACIÓN: Gaceta 593 de 27/06/2008, N° de publicación 559

**PRIORIDAD: US 60/607,834 de 08/09/2004
US 60/666,550 de 30/03/2005**

SOLICITANTE: GENENTECH, INC.

APODERADO: JUAN PABLO CADENA SARMIENTO.

TRÁMITE: SUSTENTACION DE OPOSICIÓN

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **LABORATORIO FRANCO-COLOMBIANO, LAFRANCOL S.A.**, dentro del trámite de la referencia, en ejercicio de lo dispuesto por el artículo 42 inciso 2° de la Decisión 486 de 2000, por medio del presente escrito, procedo a sustentar la oposición presentada oportunamente ante ese Despacho, en los siguientes términos:

I. COMPLEMENTO DE INFORMACIÓN

Comendidamente me permito presentar los argumentos que demuestran que la solicitud de la referencia incumple los requisitos de patentabilidad establecidos por los artículos 14, 16, 18 y 20 literal d) de la Decisión 486 de 2000, y que por ello no merece recibir el beneficio patentario.

La solicitud colombiana **07-023-376** que reivindica un tratamiento terapéutico, que involucra combinar un anticuerpo de receptor de muerte celular y un anticuerpo CD20, para así tratar la proliferación de células cancerosas tipo linfoma o una enfermedad inmunorelacionada, anticuerpos obtenidos a través de una célula hospedera recombinante; carece de novedad y nivel inventivo como se argumenta a continuación:

- ❖ En primera instancia, todas las reivindicaciones de dicha solicitud colombiana en trámite incurren en una improcedencia directa de patentabilidad, en cuanto a que explícitamente se refieren a un tratamiento terapéutico, que involucra combinar un anticuerpo de receptor de muerte celular y un anticuerpo CD20, para así tratar la proliferación de células cancerosas tipo linfoma o una enfermedad inmunorelacionada; lo cual va en contra de lo expuesto por la decisión 486 de 2000 que explícitamente revela en su artículo 20 literal d) *"No serán patentables (...) los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales."*
- ❖ En segundo lugar, y tal como lo expresa la solicitud colombiana en trámite en su reivindicación 12, donde se menciona explícitamente que dichos anticuerpos de receptor de muerte celular y anticuerpo CD20, se expresan en una célula hospedera recombinante; lo cual no resulta novedoso ni mucho menos aplica algún nivel inventivo, si se tiene en cuenta que el ADN recombinante, o ADN recombinado, es una técnica ampliamente reconocida en el estado de la técnica, y consiste en formar una molécula de ADN por la unión de dos moléculas heterólogas, es decir, de diferente origen. Como cualquier persona medianamente versada en la materia reconoce, generalmente se le aplica este nombre a moléculas producidas esta técnica, es decir a moléculas obtenidas por la unión artificial y deliberada, in vitro, de ADN proveniente de dos organismos diferentes, que normalmente no se encuentran juntos. Se reconoce ampliamente también que al introducirse este ADN recombinante en un organismo se produce una modificación genética que permite la adición de una nueva característica al organismo hospedero, conllevando a la modificación de rasgos existentes o la expresión de nuevos rasgos. Por ejemplo, y como cualquier persona medianamente versadamente en la materia reconoce, la producción de una proteína no presente inicialmente en el organismo

hospedero es posible a partir de esta técnica, y a dichas proteínas producidas a partir de dicho ADN recombinante (foráneo) se les denomina comúnmente proteínas recombinantes. Este proceso se lleva a cabo mediante técnicas de ingeniería dirigida (ingeniería genética) cuyas características, elementos y condiciones necesarias para su aplicación se encuentran también ampliamente descritas tal y como lo demuestra todo lo contenido en la publicación de carácter científico denominada "ADN Recombinante"¹. En el mismo sentido se pueden obtener, además, una gran cantidad de documentos de carácter científico en donde se aplica dicha técnica de manera muy versátil para obtener una gran cantidad de resultados^{2,3}. Siendo este el caso de la solicitud colombiana en trámite, donde reivindica la obtención de dichos anticuerpos a través de esta técnica (ADN recombinante) resulta irrefutablemente evidente la carencia de nivel inventivo en este aspecto.

- ❖ Por otro lado, y tratando el aspecto referido a la combinación farmacológica entre anticuerpos de muerte celular y un anticuerpo CD20 (Rituximab) , existe una gran cantidad de información científica, que en combinación, conllevaría a cualquier persona medianamente versada en la materia a concluir que dicha combinación entre estos anticuerpos en una formulación farmacéutica, sería útil en el tratamiento de trastornos tipo esclerosis múltiple y/o artritis reumatoide, veamos:

CD20-Positive Peripheral T-Cell Lymphoma: Report Of A Case After Nodular Sclerosis Hodgkin's Disease And Review Of The Literature⁴.

Abstract: *We present a case of peripheral T-cell lymphoma co-expressing CD3 and CD20, as well as demonstrating T-cell receptor gene rearrangement, in a patient who had been diagnosed with nodular sclerosis Hodgkin's disease 5 years previously. Although 15 cases of CD20-positive T-cell neoplasms have been previously reported in the literature, this is the first report of CD20-positive T-cell lymphoma occurring subsequent to treatment of Hodgkin's disease. The current case affords an opportunity to review the rarely reported expression of CD20 in T-cell neoplasms as well as the relationship between Hodgkin's disease and subsequently occurring non-Hodgkin's lymphomas. In addition, the identification of this case supports the suggestion that the use of CD20*

¹ Lic. Marcelo F. Goyanes. Biología Molecular y Celular.
http://www.korion.com.ar/archivos/adn_recombinante.pdf
² <http://www.sinab.unal.edu.co:2080/muse/servlet/MusePeer>
³ <http://www.sinab.unal.edu.co:2080/muse/servlet/MusePeer>
⁴ Mohrmann RL, Arber DA. Mod Pathol. 2000 Nov;13(11):1244-52.Click here to read.

antibodies alone in paraffin sections may lead to an incorrect determination of cell lineage in some cases.

Efficacy Of B-Cell-Targeted Therapy With Rituximab In Patients With Rheumatoid Arthritis⁵.

Abstract: **BACKGROUND:** An open-label study indicated that selective depletion of B cells with the use of rituximab led to sustained clinical improvements for patients with rheumatoid arthritis. To confirm these observations, we conducted a randomized, double-blind, controlled study. **METHODS:** We randomly assigned 161 patients who had active rheumatoid arthritis despite treatment with methotrexate to receive one of four treatments: oral methotrexate ($> \text{ or } = 10 \text{ mg per week}$) (control); rituximab (1000 mg on days 1 and 15); rituximab plus cyclophosphamide (750 mg on days 3 and 17); or rituximab plus methotrexate. Responses defined according to the criteria of the American College of Rheumatology (ACR) and the European League against Rheumatism (EULAR) were assessed at week 24 (primary analyses) and week 48 (exploratory analyses). **RESULTS:** At week 24, the proportion of patients with 50 percent improvement in disease symptoms according to the ACR criteria, the primary end point, was significantly greater with the rituximab-methotrexate combination (43 percent, $P=0.005$) and the rituximab-cyclophosphamide combination (41 percent, $P=0.005$) than with methotrexate alone (13 percent). In all groups treated with rituximab, a significantly higher proportion of patients had a 20 percent improvement in disease symptoms according to the ACR criteria (65 to 76 percent vs. 38 percent, $P< \text{ or } =0.025$) or had EULAR responses (83 to 85 percent vs. 50 percent, $P< \text{ or } =0.004$). All ACR responses were maintained at week 48 in the rituximab-methotrexate group. The majority of adverse events occurred with the first rituximab infusion: at 24 weeks, serious infections occurred in one patient (2.5 percent) in the control group and in four patients (3.3 percent) in the rituximab groups. Peripheral-blood immunoglobulin concentrations remained within normal ranges. **CONCLUSIONS:** In patients with active rheumatoid arthritis despite methotrexate treatment, a single course of two infusions of rituximab, alone or in combination with either cyclophosphamide or continued methotrexate, provided significant improvement in disease symptoms at both weeks 24 and 48. Copyright 2004 Massachusetts Medical Society

Association of HLA-DR5 (DR11) with systemic sclerosis (scleroderma) in Mexican patients⁶.

Abstract: **OBJECTIVE:** To study the association between Major Histocompatibility Complex (MHC) haplotypes and systemic sclerosis (SSc) in Mexican mestizo patients. **METHODS:** Class I, II and III MHC antigens were determined in 41 Mexican mestizo patients with SSc, 113 of their first degree

⁵ Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, Stevens RM, Shaw T. N Engl J Med. 2004 Jun 17;350(25):2572-81. Click here to read.
⁶ Vargas-Alarcón G, Granados J, Ibañez de Kasep G, Alcocer-Varela J, Alarcón-Segovia D. Clin Exp Rheumatol. 1995 Jan-Feb;13(1):11-6.

relatives, and 85 ethnically matched controls. The significance of differences between patients and controls was tested by chi-square analysis with Yates' correction. **RESULTS:** Frequencies of HLA-DR5 and HLA-DRw52 were found to be higher in SSc patients compared to ethnically matched healthy controls ($p = 0.007$, $RR = 3.31$; 95% confidence interval: 1.3-8.3 and $p = 0.04$, $RR = 2.4$; 95% confidence interval: 1.0-5.7, respectively). Sequence-specific oligotyping in DR5 positive individuals showed that 10 out of 41 patients had the DRB1*1104 subtype (24.3%) as compared to only 6 of the 85 healthy controls (7.0%) ($p = 0.01$, $RR = 4.25$). Subdividing patients according to their clinical features showed a significant increase of HLA-DR5 in diffuse ($p = 0.013$, $RR = 3.89$, 95% confidence interval: 1.27-12.0) and limited scleroderma ($p = 0.0008$), but not in the CREST syndrome. Segregation analysis obtained from the families showed that in the patients, DR5 was mostly part of the [HLAB35;DR5] haplotype as opposed to healthy controls. **CONCLUSION:** These data support the role of DR5 (DRB1*1104) in the genetic susceptibility to develop scleroderma in Mexican patients and also sustain the notion of genetically determined clinical subgroups of SSc.

TRAIL-R2 (DR5) mediates apoptosis of synovial fibroblasts in rheumatoid arthritis⁷.

Abstract: TRAIL has been proposed as an anti-inflammatory cytokine in animal models of rheumatoid arthritis (RA). Using two agonistic mAbs specific for TRAIL-R1 (DR4) and TRAIL-R2 (DR5), we examined the expression and function of these death receptors in RA synovial fibroblast cells. The synovial tissues and primary synovial fibroblast cells isolated from patients with RA, but not those isolated from patients with osteoarthritis, selectively expressed high levels of cell surface DR5 and were highly susceptible to anti-DR5 Ab (TRA-8)-mediated apoptosis. In contrast, RA synoviocytes did not show increased expression of TRAIL-R1 (DR4), nor was there any difference in expression of Fas between RA and osteoarthritis synovial cells. In vitro TRA-8 induced apoptosis of RA synovial cells and inhibited production of matrix metalloproteinases induced by pro-inflammatory cytokines. In vivo TRA-8 effectively inhibited hypercellularity of a SV40-transformed RA synovial cell line and completely prevented bone erosion and cartilage destruction induced by these cells. These results indicate that increased DR5 expression and susceptibility to DR5-mediated apoptosis are characteristic of the proliferating synovial cells in RA. As highly proliferative transformed-appearing RA synovial cells play a crucial role in bone erosion and cartilage destruction in RA, the specific targeting of DR5 on RA synovial cells with an agonistic anti-DR5 Ab may be a potential therapy for RA.

Como se puede apreciar, aunque no se mencione en éstas publicaciones explícitamente la combinación de dichos anticuerpos, la información que allí se incluye en donde se relacionan los objetivos terapéuticos de dichos

⁷ Ichikawa K, Liu W, Fleck M, Zhang H, Zhao L, Ohtsuka T, Wang Z, Liu D, Mountz JD, Ohtsuki M, Koopman WJ, Kimberly R, Zhou T. J Immunol. 2003 Jul 15;171(2):1061-9.

anticuerpos, obviamente es una guía para asociar dichos agentes proteicos para tratar dichos trastornos relacionados con sus moléculas objetivo moléculas sobre las que funcionan como anticuerpos.

❖ Por su parte la publicación internacional WO 03/038043 publicada el 08 de mayo de 2003 y titulada "COMBINATIONS OF ANTIBODIES SELECTIVE FOR A TUMOR NECROSIS FACTOR RELATED APOPTOSIS INDUCING LIGAND RECEPTOR AND OTHER THERAPEUTIC AGENTS" revela:

1. A method of selectively inducing apoptosis in target cells expressing DR5, comprising the steps of (a) contacting the target cells with a therapeutic quantity of an antibody that specifically binds a TRAIL receptor DR5, wherein said antibody, in its soluble form, has *in vivo* and *in vitro* apoptosis-inducing activity in target cells expressing DR5 and (b) contacting the target cells with a therapeutic quantity of one or more therapeutic agents.
2. The method of claim 1, wherein at least one contacting step is performed *in vivo*.
(...)
4. The method of claim 1, wherein the therapeutic agent or agents are chemotherapeutic agents.
(...)
8. The method of claim 7, further comprising administering rituximab.
(...)
23. The method of claim 1, wherein the therapeutic agent or agents are disease modifying anti-rheumatic drugs.
(...)
27. The method of claim 1, wherein the therapeutic agent is an apoptosis-inducing compound.
(...)

30. The method of claim 29, wherein the second antibody is selected from the group consisting of a DR4 antibody, a TNF antibody, a B7 antibody, a CD40 ligand antibody, a CD40 antibody, a CD20 antibody, and a Fas antibody.

(...)

32. The method of claim 1, wherein the target cell is an abnormally proliferating synovial cell.

33. The method of claim 32, wherein the synovial cell is a rheumatoid arthritis synovial cell.

(...)

39. A method of inhibiting proliferation of target cells expressing DR5, comprising the steps of (a) contacting the target cells with a therapeutic quantity of an antibody that specifically binds a TRAIL receptor DR5, wherein said antibody, in its soluble form, has *in vivo* and *in vitro* apoptosis-inducing activity in cells expressing DR5 and (b) contacting the target cells with a therapeutic quantity of one or more therapeutic agents.

(...)

46. The method of claim 45, further comprising administering rituximab.

(...)

66. The method of claim 65, wherein the second antibody is selected from the group consisting of a DR4 antibody, a TNF antibody, a B7 antibody, a CD40 ligand antibody, a CD40 antibody, a CD20 antibody, and a Fas antibody.

67. The method of claim 1, wherein the therapeutic agent is an apoptosis-inducing compound.

(...)

106. The method of claim 77, wherein the inflammatory or autoimmune disease is selected from the group consisting of systemic lupus erythematosus, Hashimoto's disease, rheumatoid arthritis, graft-versus-host disease, Sjögren's syndrome, pernicious anemia, Addison disease, scleroderma, Goodpasture's syndrome, Crohn's disease, autoimmune hemolytic anemia, sterility, myasthenia gravis, multiple sclerosis, Basedow's disease, thrombotic thrombocytopenia, thrombopenia purpura, insulin-dependent diabetes mellitus, allergy; asthma, atopic disease; arteriosclerosis; myocarditis; cardiomyopathy; glomerular nephritis; hypoplastic anemia.

107. The method of claim 77, further comprising administering to the subject radiation therapy.

(...)

110. The composition of claim 108, further comprising a pharmaceutically acceptable carrier.

Como se puede apreciar claramente, en esta anterioridad se revela en esencia todos los aspectos que fundamentan tanto la novedad como el nivel inventivo de la solicitud colombiana en tramite; específicamente en lo que se refiere a la combinación de anticuerpos re receptor de muerte celular y un anticuerpo anti-CD20, específicamente anticuerpos de unión a DR5 y DR4 junto con Rituximab, en donde opcionalmente dicho método puede usar radiación y resulta útil para elaborar formulaciones farmacéuticas para el tratamiento de enfermedades proliferativas, artritis reumatoidea y esclerosis múltiple.

Finalmente existen otro documentos que así mismo revelan la obtención de anticuerpos del mismo tipo que los mencionados por la solicitud colombiana en tramite (anti-DR4, anti-DR5, anti CD-20) en algunas combinaciones o tratamientos que resultan ser información muy clara y que sirve como argumento adicional contra la novedad y el nivel inventivo de la solicitud colombiana en tramite. Algunos de estos documentos pueden ser la publicación internacional WO 01/77342 publicada el 18 de Octubre de 2001 y la publicación internacional WO 99/37684 publicada el 29 de Julio de 1999.

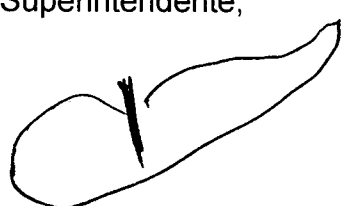
Por todo lo anterior reitero que la solicitud de la referencia, en todas sus reivindicaciones, incumple los requisitos materiales de patentabilidad establecidos

por la Decisión 486 de 2000, razón por la cual no puede ser objeto de privilegio de patente de invención.

VI. ANEXOS

- ❖ Copia completa del documento Lic. Marcelo F. Goyanes. Biología Molecular y Celular. http://www.korion.com.ar/archivos/adn_recombinante.pdf
- ❖ Copias de la búsqueda en el metabuscador de la página de La Universidad Nacional de Colombia bajo los términos "ADN recombinante" y "Recombinant DNA".
- ❖ La publicación internacional WO 03/038043 publicada el 08 de mayo de 2003 y titulada "COMBINATIONS OF ANTIBODIES SELECTIVE FOR A TUMOR NECROSIS FACTOR RELATED APOPTOSIS INDUCING LIGAND RECEPTOR AND OTHER THERAPEUTIC AGENTS" capítulo reivindicatorio.
- ❖ Capítulos reivindicatorios de la publicación internacional WO 01/77342 publicada el 18 de Octubre de 2001 y la publicación internacional WO 99/37684 publicada el 29 de Julio de 1999.

Señor Superintendente,



JOSÉ LUIS REYES VILLAMIZAR

C.C. 79'152.473 de Usaquén

T.P.A 44.655 del C.S.J.

ADN Recombinante

Hasta las postrimerías de 1970, el ADN era una molécula que presentaba amplias dificultades para su análisis bioquímico. Su gran longitud y monotonía hacían que la secuencia de nucleótidos pudiese sólo ser estudiada mediante mecanismos indirectos. Para esto se recurría a la determinación de la secuencia de las proteínas o del ARN.

Hoy la situación ha cambiado por completo y el ADN ha pasado a ser una de las moléculas más fáciles de estudiar. Mediante las técnicas que desarrollaremos en este capítulo, veremos que es factible separar regiones determinadas del ADN, obtenerlas en grandes cantidades y determinar su secuencia de nucleótidos a una velocidad de varios cientos de nucleótidos al día. También se nos hace posible alterar un gen (sometido a ingeniería) y transferirlo a células en cultivo o a la línea germinal de animales, donde el gen modificado se incorpora como parte funcional y permanente del genoma.

La tecnología del ADN recombinante constituye una suma de técnicas siendo las más importantes:

- ✓ La *rotura específica del ADN mediante nucleasas de restricción*, que facilita el aislamiento y la manipulación de los genes individuales.
- ✓ La *secuenciación rápida* de todos los nucleótidos de un fragmento purificado de ADN, que posibilita determinar los límites de un gen y la secuencia de aminoácidos que codifica.
- ✓ La *hibridación* de los ácidos nucleicos que hace posible localizar secuencias determinadas de ADN o ARN, utilizando la capacidad que tienen estas moléculas de unirse a secuencias complementarias de otros ácidos nucleicos.
- ✓ La *clonación del ADN*, mediante la cual se puede conseguir que un fragmento de ADN se integre en un elemento génico autorreplicante (plásmido o virus) que habita en una bacteria, de tal manera, que una molécula simple de ADN puede ser producida generando muchos miles de millones de copias idénticas.
- ✓ La *ingeniería genética* mediante la cual se pueden alterar secuencias de ADN produciendo versiones modificadas de los genes, los cuales se pueden insertar a células u organismos.

Fragmentación, separación y secuenciación de moléculas de ADN

A diferencia de las proteínas, los genes no existen en unidades independientes, sino que confieren regiones de una molécula de ADN de gran tamaño. Aunque es posible fragmentar el ADN al azar, es muy difícil que estas porciones contengan un determinado gen. Así, el purificar un determinado gen constituyó un verdadero problema hasta la aparición de las endonucleasas de restricción. Estas enzimas, que pueden aislarse de bacterias, cortan el ADN de doble cadena en lugares discretos, definidos por la secuencia de nucleótidos, produciendo fragmentos de ADN de tamaño definido. Distintas especies de bacterias fabrican diferentes endonucleasas de restricción y cada una de ellas posee especificidades de secuencia, siendo relativamente fácil encontrar una endonucleasa de restricción que libere un fragmento de ADN que incluya un determinado gen. El tamaño del fragmento liberado puede utilizarse para purificar el gen de una mezcla. Una vez purificado, puede amplificarse el fragmento de ADN

mediante clonación y determinarse la secuencia de nucleótidos que se encuentran en la molécula mediante la secuenciación.

Las endonucleasas de restricción son producidas por un amplio número de bacterias, a las que las protegen degradando moléculas de ADN que han sido transportadas al interior de las mismas por medio de virus. Así, una bacteria puede eliminar una porción de ácido nucleico viral que ha ingresado a su genoma.

Cada enzima reconoce una secuencia específica de ADN de entre cuatro y ocho nucleótidos. Cuando estas secuencias son propias del genoma bacteriano están protegidas del corte por medio de la metilación; cuando estas secuencias se presentan en un ADN extraño, no se hallan metiladas por lo cual resultan como blanco del ataque de las endonucleasas. Se han aislado y purificado muchas nucleasas de restricción de diferentes especies bacterianas y actualmente existen más de cien de estas enzimas en el mercado.

Algunas de las endonucleasas de restricción cortan el ADN generando secuencias cortas de ADN de cadena sencilla en los extremos del fragmento. A este tipo de extremos se los conoce como "adhesivos o cohesivos", pues es complementario de otra secuencia que genera la misma endonucleasa en otro fragmento. Los extremos cohesivos permiten unir fácilmente fragmentos de ADN obtenidos con la misma enzima.

Las moléculas de ADN producidas mediante la unión de dos o más fragmentos de ADN se denominan **Moléculas de ADN Recombinantes**.

Una determinada endonucleasa de restricción cortará cualquier doble hélice de ADN de una determinada célula, generando una serie de fragmentos de ADN conocidos como Fragmentos de Restricción. Si se combinan diferentes enzimas de restricción, se pueden comparar los fragmentos obtenidos construyendo así un mapa de restricción que muestre la localización de cada punto de corte en relación con los puntos de restricción vecinos. Un mapa de restricción refleja la disposición de secuencias de nucleótidos específicas en la región elegida para el corte. Esto significa que es posible realizar la comparación de diferentes regiones de ADN, sin tener que determinar sus secuencias de nucleótidos. Se pueden comparar así los mapas de restricción de ADN humano y de varios primates en un grupo de genes que codifican para una misma proteína. Si esto se realiza en aquellos genes que codifican para la hemoglobina, se podrá observar que en el hombre, el orangután y el chimpancé, las regiones codificantes han permanecido constantes durante los 5 a 10 millones de años que los separan en el camino evolutivo.

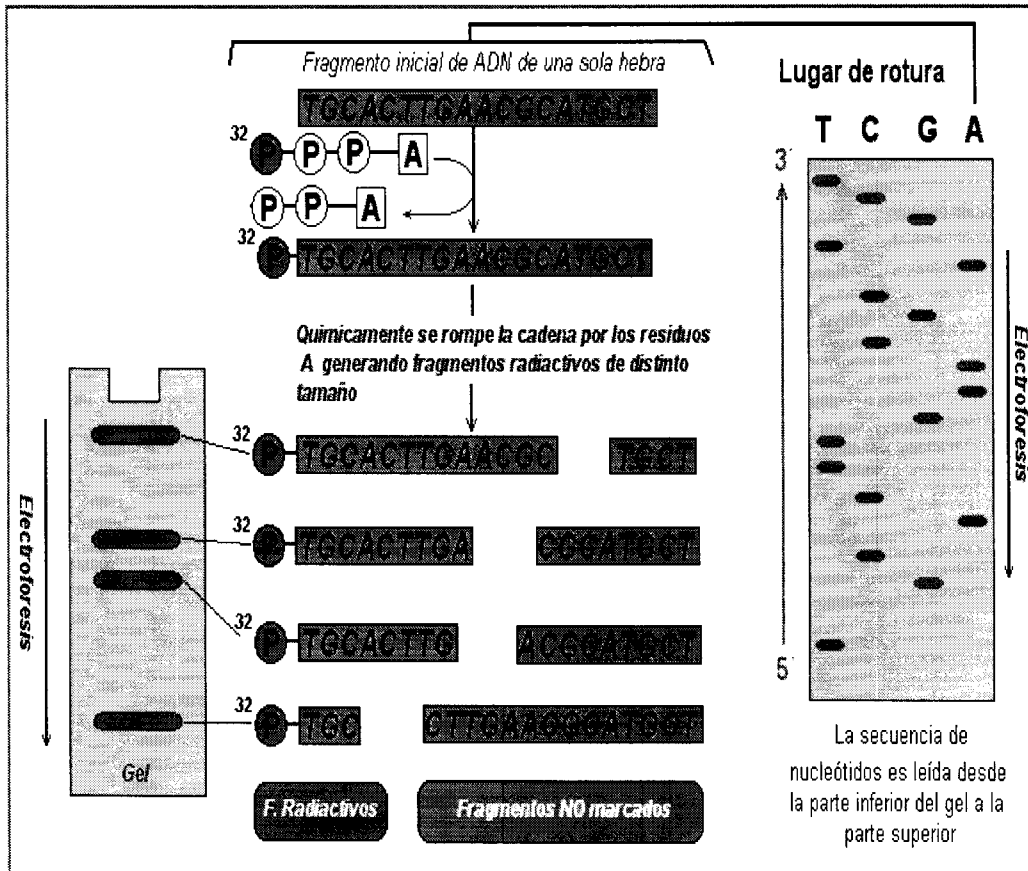
Los mapas de restricción son también importantes para la clonación de ADN y para la ingeniería genética, permitiendo localizar un gen de interés en un fragmento determinado, facilitando así su aislamiento.

Secuenciación de los fragmentos de ADN

Existen dos métodos específicos para determinar la secuencia de nucleótidos de cualquier fragmento de ADN purificado: el método químico y el enzimático.

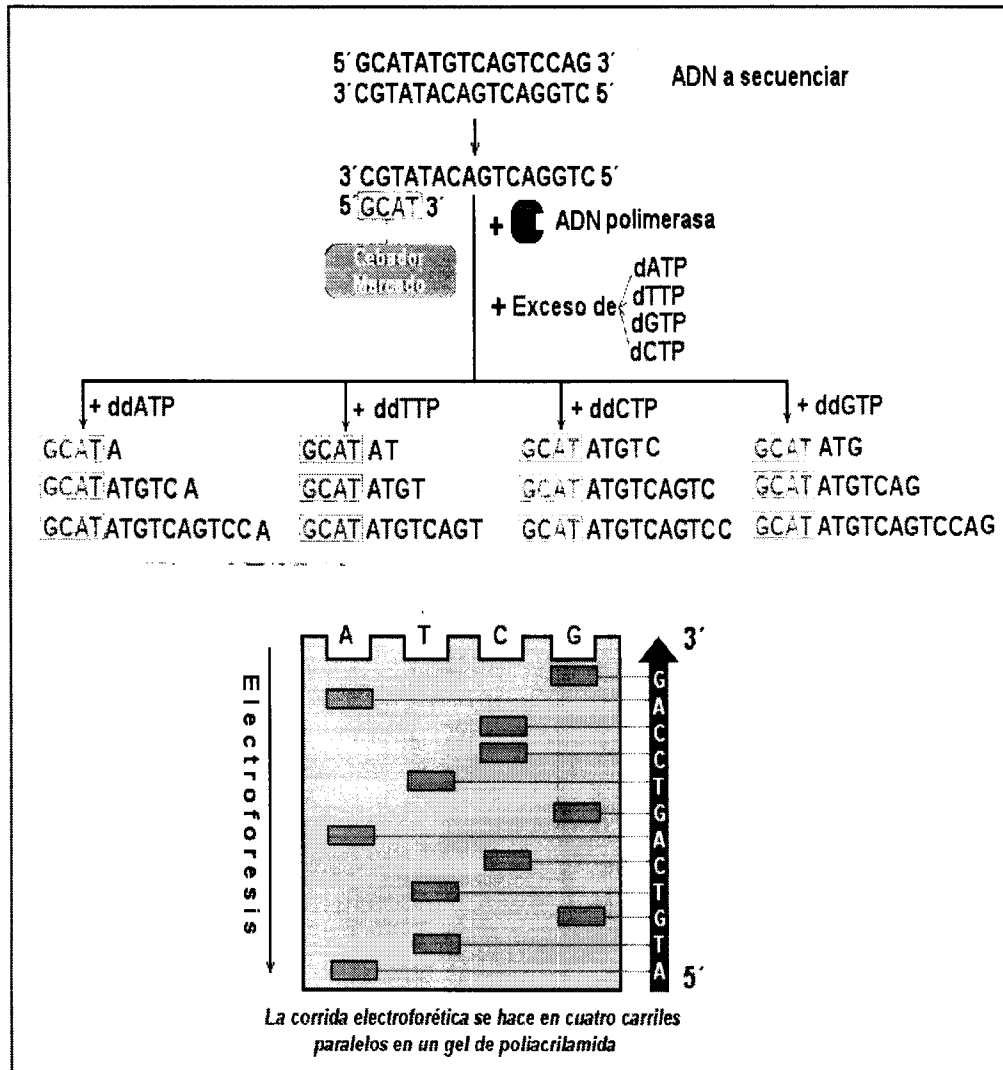
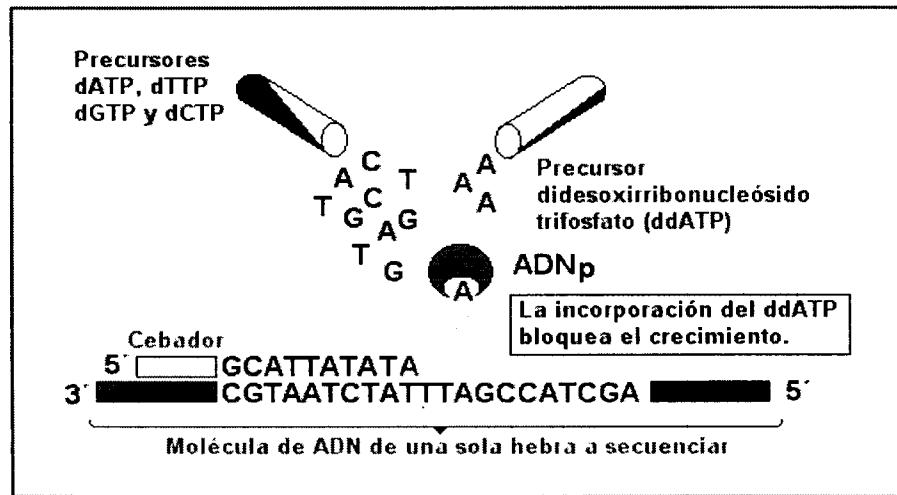
El método químico de secuenciación se inicia con la obtención de un conjunto de moléculas de ADN de doble cadena marcadas en un extremo 5' mediante la acción de la polinucleótido quinasa y ATP marcado con ^{32}P . Luego, las cadenas de la **doble** hélice de ADN se disocian y se someten a un tratamiento suave con un agente químico que destruye una de las cuatro bases nitrogenadas. Debido a la suavidad del tratamiento, sólo se rompe, al azar, una base por molécula. Este tratamiento genera una suma de

fragmentos de ADN de diferentes longitudes, que reflejan los lugares donde se encontraban las bases que fueron eliminadas. Los fragmentos son separados mediante la utilización de un gel y se detectan por autorradiografía. Es necesario mencionar que sólo los fragmentos que poseen un extremo 5' terminal ^{32}P aparecen en el gel y su tamaño indica la distancia desde el extremo marcado hasta la base nitrogenada previamente destruida.



En el método enzimático de secuenciación, el ADN es utilizado como molde de la ADN polimerasa para obtener una serie de réplicas parciales, que comienzan siempre en el mismo sitio pero que terminan en diferentes puntos a lo largo de la cadena de ADN. Este método se basa en la utilización de dideoxirribonucleótidos trifosfatados que, al carecer del grupo oxidrilo en el extremo 3', impiden el correspondiente agregado de más nucleótidos a la cadena de ADN en formación. Esta reacción genera, por tanto, una escalera de fragmentos que pueden ser separados mediante la utilización de la electroforesis en gel de poliacrilamida. Los fragmentos son detectados por la presencia de un marcador químico o radiactivo que puede ser incorporado, tanto en el oligonucleótido, como en los desoxirribonucleótidos usados para extender la cadena de ADN. Con lo descrito hasta el momento, sería imposible realizar la secuenciación de la molécula de ADN. Pero, si se utilizan los cuatro nucleósidos trifosfatos terminadores de cadena en cuatro reacciones diferentes y los productos de los mismos se hacen correr en cuatro carriles paralelos, la secuencia de ADN puede deducirse como en el método químico.

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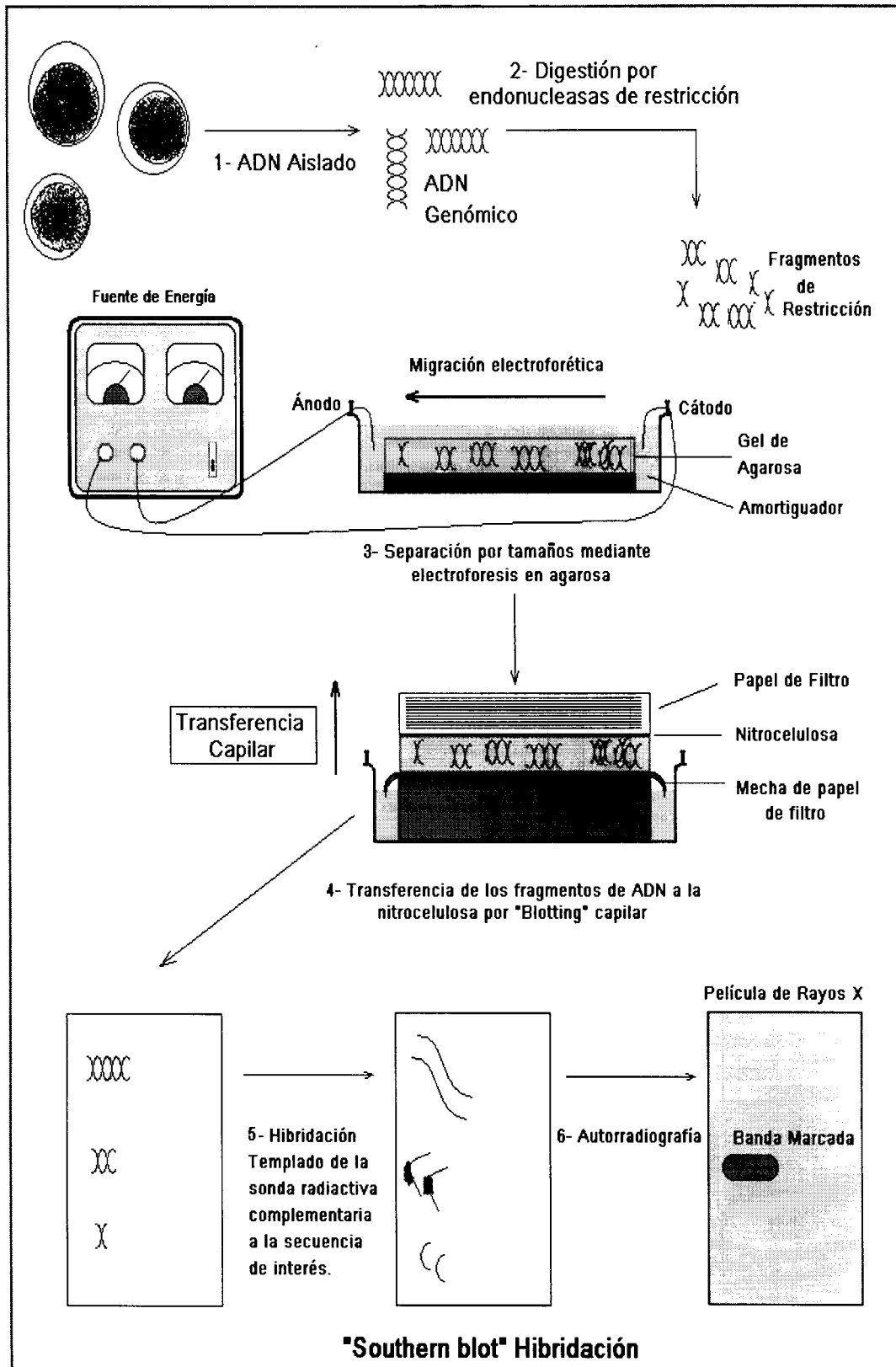


Fragmentos de restricción, electroforesis y métodos de transferencia

Los fragmentos de restricción pueden ser separados por electroforesis en un gel de agarosa sobre la base de sus tamaños. Ello posibilita la localización específica de uno de los fragmentos, para lo cual se comienza tratando al gel con una solución alcalina que desnaturalice las moléculas de ADN, es decir, separe sus dos cadenas. Con el objeto de facilitar la manipulación de las bandas electroforéticas (invisibles para el investigador), éstas son transferidas a un filtro de nitrocelulosa. Para ello el gel se coloca sobre varias hojas de papel empapadas con solución fisiológica, se aplica el filtro de nitrocelulosa sobre el gel. Y sobre el filtro se ponen varias hojas secas de papel absorbente. Dado que la solución salina asciende por capilaridad desde las hojas empapadas hacia las secas, arrastra a los fragmentos de ADN contenidos en las bandas del gel, las cuales son transferidas al filtro de nitrocelulosa y quedan localizadas en las posiciones que tenían en el gel. El filtro separado del gel y de los papeles usados para la transferencia del ADN, se trata con una sonda radiactiva de ADNc (marcada con ^{32}P), específica para el segmento de ADN que interesa localizar. Tras la hibridación de ambos ADN, el filtro. Se aplica sobre una película fotográfica, de modo que la o las bandas que contienen el ADN buscado queden impresas en ella (radioautografía). Para localizar los segmentos de ADN en el gel de agarosa, bastará confrontarlo con la película.

La transferencia del ADN desde el gel de agarosa al filtro de nitrocelulosa lleva el nombre de Southern blotting, en parte por su significado literal (blotting, transferencia) y en parte como homenaje al creador del método, E. M. Southern. Existen técnicas semejantes para transferir ARN y proteínas, designadas -no sin humor- Northern blotting y Western blotting, respectivamente.

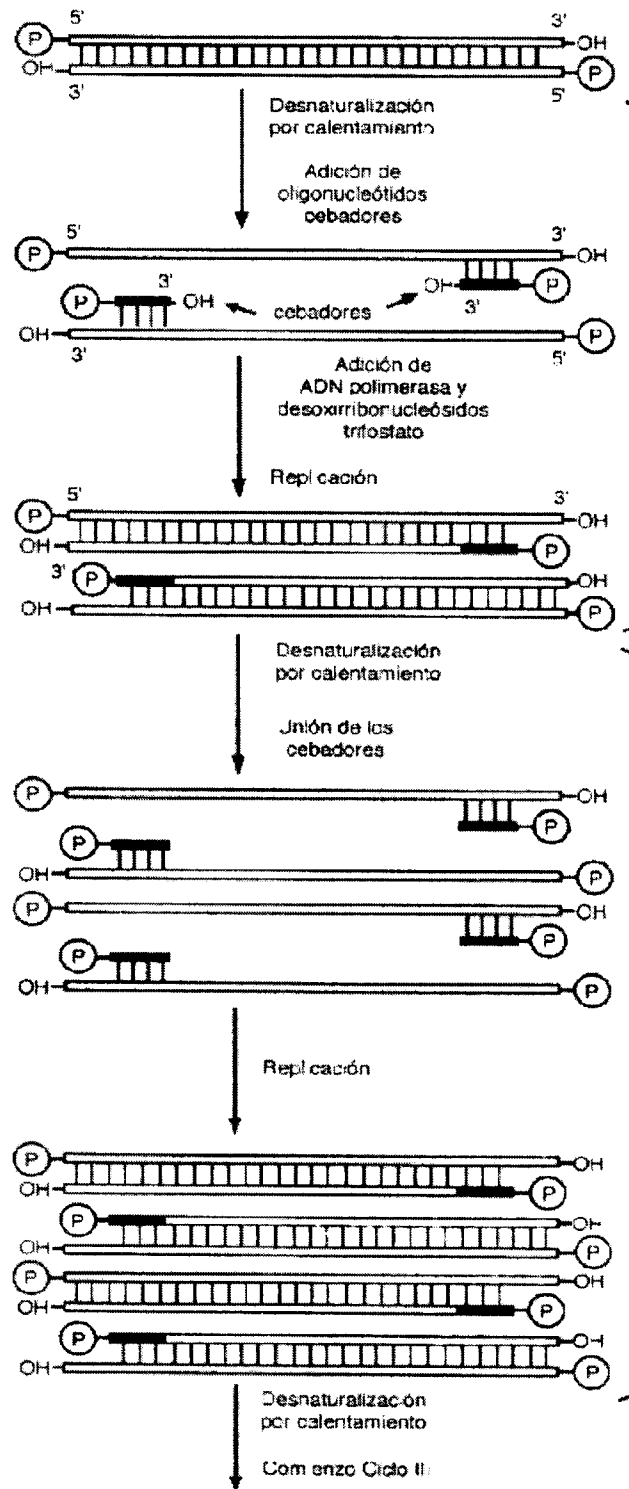
15
457



460
18

Detección y amplificación de secuencias por el Método de la reacción en cadena de la polimerasa (PCR).

Si una región de ADN ya se ha clonado y secuenciado, la secuencia puede utilizarse para obtener partes de esa región a partir de individuos concretos de esa o de otra especie, sin necesidad de clonarlos. La técnica se basa en un procedimiento denominado reacción en cadena de la polimerasa (PCR, del inglés «polymerase chain reaction»). Para catalizar la extensión de los cebadores, se utiliza una polimerasa de ADN termoestable. La polimerasa Taq, obtenida a partir de la bacteria *Thermus aquaticus*. La polimerasa extiende en sentido convergente a partir de dos cebadores que hibridan con cadena opuesta. Una vez completada la replicación del segmento delimitado por los dos cebadores (primer ciclo), las dos moléculas, nuevas de doble cadena se desnaturalizan por calor y se procede a un segundo ciclo de replicación, tras bajar la temperatura en presencia de todos los componentes necesarios para la polimerización. La repetición de los ciclos de interés y desnaturalización lleva a un aumento exponencial del número de segmentos replicados. Resulta fácil conseguir niveles de amplificación de hasta un millón. La utilización de la técnica de la PCR permite la amplificación de un gen de copia única a partir de una muestra genómica, siempre que podamos sintetizar cebadores complementarios a secuencias conocidas del gen. Debido a la naturaleza exponencial de la amplificación, la PCR es una técnica muy sensible, que permite detectar secuencias mínimamente representadas en una muestra. Por ejemplo, se pueden amplificar segmentos de ADN humano a partir de las pocas células foliculares que rodean a un solo pelo. La PCR resulta muy útil en el diagnóstico del ADN en otras palabras, en la comprobación de la presencia de un gen o de una mutación en un gen específico o, al nivel preparativo, en la amplificación de un segmento determinado. Advierta que la técnica de la PCR no requiere la digestión del sustrato con enzimas de restricción, ya que los cebadores se encargarán de encontrar la secuencia adecuada en el ADN nativo. Además, podemos prescindir de experimentos de clonación tediosos, porque se sintetiza suficiente ADN para producir una banda nítida en un gel. Aún más, se requieren cantidades ínfimas del ADN sustrato. La reacción en cadena de la polimerasa utiliza cebadores diseñados especialmente para amplificar una región concreta de ADN sin necesidad de clonarlo.



CICLO I

CICLO II

Dos ciclos de la reacción en cadena de la polimerasa

19
461

Lista de resultados combinados | PubMed (0) | EBM Reviews, Cochrane Database (0) | Evidence Based Medicine Review - EBM (0) | Medline - OVID (0) | OVID - Journals@Ovid (0) | GeoRef (0) | American Chemical Society - ACS (0) | Google Scholar (27) | FindArticles (Free Articles Only) (0) | MEDLINE with Full Text (0)

☐ 1Registro Completo  

Título: ADN Recombinant, 2 ème edition

Autores: G Watson; Z Witkowski

En: Google Scholar

☐ 2Registro Completo  

Título: JM, adn ALBELDA, SM (1995). ICAM-1 expression on bronchial epithelium after recombinant adenovirus ...

Autores: PE EWSKI; S JM; W DJ

En: Google Scholar

☐ 3Registro Completo  

Título: PROCEDES DE PRODUCTION A GRANDE ECHELLE DE MOLECULES K2S TPAOR DERIVEES D'ADN RECOMBINANT

Autores: J MANOSROI; A MANOSROI; C TAYAPIWATANA; FBH GOETZ

En: Google Scholar

☐ 4Registro Completo  

Título: ... A CODIFIED POLIPEPTID BY THE ADN OF A VEHICLE OR VECTOR OR CLONATION OF RECOMBINANT ADN AND OF THE ...

Autores: PT Patent 84; 018; 1987

En: Google Scholar

☐ 5Registro Completo  

Título: VARIANT DE LUCIFERASE, GENE DE VARIANT DE LUCIFERASE, ADN RECOMBINANT ET TECHNIQUE PERMETTANT DE ...

En: Google Scholar

☐ 6Registro Completo  

Título: ADN RECOMBINANT CODANT LE PRINCIPAL ALLERGENE DU POLLEN PLANTAGO LANCEOLATA (PLA L 1) ET ...

Autores: B FREILE; A PERALES; C Díaz; D HERNÁNDEZ; G DURÁN

En: Google Scholar

☐ 7Registro Completo  

Título: VECTEUR VIRAL ET ADN RECOMBINANT CODANT POUR LA PROTEINE P25 DU VIRUS AGENT CAUSAL DU SIDA, CULTURE ...

Autores: PT Patent 86; 234; 1987

En: Google Scholar

☐ 8Registro Completo  

Título: PROCEDES DE PRODUCTION A GRANDE ECHELLE DE MOLECULES K2S TPAOR DERIVEES D'ADN RECOMBINANT

Autores: RGHHS WERNER; FBH GOETZ; C TAYAPIWATANA

En: Google Scholar

☐ 9Registro Completo  

Título: ADN recombinant et bioéthique: une application précoce du principe de précaution

Autores: JN Missa

En: Google Scholar

20
462☐ 10Registro Completo 

Título: **Contrôle de la diffusion d'ADN recombinant de champs de betteraves sucrières transgéniques par PCR**
...

Autores: P Meier; W Wackemagel

En: **Google Scholar**

☐ 11Registro Completo 

Título: ... , **Saito M** et **Nakajima Y.** **Détection de fragments d'ADN de maïs intrinsèque et recombinant et de la**
...

Autores: EH Chowdhury; H Kuribara; A Hin; P Sultana

En: **Google Scholar**

☐ 12Registro Completo 

Título: ... **de ferme: une investigation collaborative de bétail et poulets nourris avec du matériel recombinant**
...

Autores: R Einspanier; A Klotz; J Kraft; K Aulrich; R Poser

En: **Google Scholar**

☐ 13Registro Completo 

Título: ► **APLICAȚIILE ULTIMĂORĂ ÎN TEHNOLOGIA ADN-ULUI RECOMBINANT**

Autores: MM CÎMPEANU; CS CÎMPEANU; S PĂDUREANU

En: **Google Scholar**

☐ 14Registro Completo 

Título: ... **VIP (Vasoactive Intestinal Peptide)** **par les technologies de l'ADN recombinant et du génie ...**

Autores: J Raingeaud

En: **Google Scholar**

☐ 15Registro Completo 

Título: ... **analyse d'images autoradiographiques et à la représentation de cartes de molécules d'ADN recombinant**

Autores: P COUSSEAU

En: **Google Scholar**

☐ 16Registro Completo 

Título: **RECOMBINANT ADN MOLECULES CODING FOR A RECEPTOR CHAIN FOR THE T-CELL ANTIGEN, PREPARATION PROCESS, ...**

En: **Google Scholar**

☐ 17Registro Completo 

Título: **Ingeniería genética con recombinación de ADN in vitro./Genetic engineering using recombinant DNA**

Autores: L Aron Hott; F Cabello

Fuente: Rev. med. Chile, 1982

En: **Google Scholar**

☐ 18Registro Completo 

Título: **Diagnostics génétiques: par la technique de l'ADN recombinant**

Autores: H CANN

Fuente: 1985

En: **Google Scholar**

☐ 19Registro Completo 

Título: **Caractérisation du sexe à partir d'une tache de sang par les techniques d'ADN recombinant**

Autores: B LUDÉS; P MANGIN; A HANAUER; P KINTZ; A LUGNIER

463
21

Fuente: Journal de médecine légale droit médical, 1990

En: **Google Scholar**

☐ **20**

Registro Completo 

Título: **La technologie de l'ADN recombinant et la brasserie**

Autores: M LAURENT; A DEBOURG

Fuente: Cerevisia and biotechnology, 1991

En: **Google Scholar**

☐ **21**

Registro Completo 

Título: ... /**The synthetic theory of evolution challenged by genetics of the recombinant DNA and developmental ...**

Autores: O Reig

Fuente: Arch. biol. med. exp, 1991

En: **Google Scholar**

☐ **22**

Registro Completo 

Título: **ADN recombinant De Boeck-Université**

Autores: JD Watson; Gilman

Fuente: 1994

En: **Google Scholar**

☐ **23**

Registro Completo 

Título: **ADN recombinant**

Autores: JD Watson

Fuente: 1994

En: **Google Scholar**

☐ **24**

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Autores: E Pérez de Colina

Fuente: Rev. Inst. Nac. Hig, 1994

En: **Google Scholar**

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Lista de resultados combinados | PubMed (1876) | EBM Reviews, Cochrane Database (0) | Evidence Based Medicine Review - EBM (82) | Medline - OVID (2104) | OVID - Journals@Ovid (12) | GeoRef (0) | American Chemical Society - ACS (0) | Google Scholar (4650) | FindArticles (Free Articles Only) (0) | MEDLINE with Full Text (10)

☐ 1Registro Completo  

Título: **Comparative Immunogenicity in Rhesus Monkeys of DNA Plasmid, Recombinant Vaccinia Virus, and ...**

Autores: DR Casimiro; L Chen; TM Fu; RK Evans; MJ Caulfield

Fuente: Journal of Virology, 2003

En: **Google Scholar**

☐ 2Registro Completo  

Título: **Nucleotide excision repair of DNA with recombinant human proteins: definition of the minimal set of ...**

Autores: SJ Araujo; F Tirode; F Coin; H Pospiech; JE

Fuente: Genes & Development, 2000

En: **Google Scholar**

☐ 3Registro Completo  

Título: **... trials: stimulation of HIV-specific T-cell responses by DNA and recombinant modified vaccinia virus ...**

Autores: M Mwau; I Cebere; J Sutton; P Chikoti; N Winstone

Fuente: Journal of General Virology, 2004

En: **Google Scholar**

☐ 4Registro Completo  

Título: **... -Human Immunodeficiency Virus 89.6 P Challenge by a DNA Prime/Recombinant Modified Vaccinia Virus ...**

Autores: RR Amara; JM Smith; SI Staprans; DC Montefiori

Fuente: Journal of Virology, 2002

En: **Google Scholar**

☐ 5Registro Completo  

Título: **... by Components of a Tuberculosis Polyprotein Vaccine, Mtb72F, Delivered as Naked DNA or Recombinant ...**

Autores: YAW Skeiky; MR Alderson; PJ Ovendale; JA Guderian

Fuente: The Journal of Immunology, 2004

En: **Google Scholar**

☐ 6Registro Completo  

Título: **Recombinant DNA**

Autores: JD Watson; M Gilman; J Witkowski; M Zoller

Fuente: 1992

En: **Google Scholar**

☐ 7Registro Completo  

Título: **... against SHIV by Gene-Gun Delivery of HIV-1 DNA Followed by Recombinant Subunit Protein Boosts**

Autores: P Putkonen; M Quesada-Rolander; AC Leandersson

Fuente: Virology, 1998

En: **Google Scholar**

☐ 8Registro Completo  

Título: **... and CD4+ T Cell and Antibody Responses by Sequential Immunization with Malaria DNA and Recombinant ...**

Autores: R Wang; J Epstein; Y Charoenvit; FM Baraceros

Fuente: The Journal of Immunology, 2004

465₂₃*En:* **Google Scholar**☐ 9Registro Completo *Título:* ... -**Recombinant Canarypox Virus Boosting Induces Immune Responses and Protection from Recombinant HCV-** ...*Autores:* P Pancholi; M Perkus; N Tricoche; Q Liu; AM Prince*Fuente:* Journal of Virology, 2003*En:* **Google Scholar**☐ 10Registro Completo *Título:* **A heterologous prime-boost regime using DNA and recombinant vaccinia virus expressing the ...***Autores:* RM Gonzalo; G del Real; JR Rodriguez; D Rodriguez*Fuente:* Vaccine, 2002*En:* **Google Scholar**

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Lista de resultados combinados | PubMed (1876) | EBM Reviews, Cochrane Database (0) | Evidence Based Medicine Review - EBM (82) | Medline - OVID (2104) | OVID - Journals@Ovid (12) | GeoRef (0) | American Chemical Society - ACS (0) | Google Scholar (4650) | FindArticles (Free Articles Only) (0) | MEDLINE with Full Text (10)

☐ 11Registro Completo  *Título:* **Recent Activity***En:* **PubMed**☐ 12Registro Completo  *Título:* **Quality Considerations for Recombinant DNA-Derived Biological Therapeutic Products: A Control Perspective on Cytokines.***Autores:* Mire-Sluis A; Thorpe R.*Fuente:* BioDrugs. 1999 Jun;11(6):367-76.*En:* **PubMed**☐ 13Registro Completo  *Título:* **Structural study of the archaeal homologous recombinant protein complexed with DNA fragments.***Autores:* Naoe C; Tsunoda M; Nakamura KT.*Fuente:* Nucleic Acids Symp Ser (Oxf). 2004;(48):115-6.*En:* **PubMed**☐ 14Registro Completo  *Título:* **[Construction of high efficient eukaryotic expression recombinant on human hepatocyte DNA synthetic stimulated factor]***Autores:* Tang SG; Su XS.*Fuente:* Hunan Yi Ke Da Xue Xue Bao. 2003 Dec;28(6):575-8. Chinese.*En:* **PubMed**☐ 15Registro Completo  *Título:* **[Immune response induced by HIV DNA vaccine combined with recombinant adeno-associated virus]***Autores:* Liu YZ; Zhou L; Wang Q; Ye SQ; Li HX; Zeng Y.*Fuente:* Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi. 2004 Sep;18(3):251-4. Chinese.*En:* **PubMed**☐ 16Registro Completo  *Título:* **Differential gene expression in recombinant Pichia pastoris analysed by heterologous DNA microarray hybridisation.***Autores:* Sauer M; Branduardi P; Gasser B; Valli M; Maurer M; Porro D; Mattanovich D.*Fuente:* Microb Cell Fact. 2004 Dec 20;3(1):17.*En:* **PubMed**☐ 17Registro Completo  *Título:* **Induction of P815 tumor immunity by DNA-based recombinant Semliki Forest virus or replicon DNA expressing the P1A gene.***Autores:* Ni B; Lin Z; Zhou L; Wang L; Jia Z; Zhou W; Diciommo DP; Zhao J; Bremner R; Wu Y.*Fuente:* Cancer Detect Prev. 2004;28(6):418-25.*En:* **PubMed**☐ 18Registro Completo  *Título:* **Intracellular viral processing, not single-stranded DNA accumulation, is crucial for recombinant adeno-associated virus transduction.***Autores:* Hauck B; Zhao W; High K; Xiao W.*Fuente:* J Virol. 2004 Dec;78(24):13678-86.*En:* **PubMed**

467 25

☐ 19Registro Completo 

Título: [Application of exponent curve model to study the hepatitis B DNA recombinant yeast derived vaccine antibody levels.]

Autores: Luo FJ; Dong CM; Shen YG; Huang CJ.

Fuente: Zhonghua Liu Xing Bing Xue Za Zhi. 2004 Sep;25(9):805-7. Chinese.

En: PubMed

☐ 20Registro Completo 

Título: DNA and low titer, helper-free, recombinant AAV prime-boost vaccination for cytomegalovirus induces an immune response to CMV-pp65 and CMV-IE1 in transgenic HLA A*0201 mice.

Autores: Gallez-Hawkins G; Li X; Franck AE; Thao L; Lacey SF; Diamond DJ; Zaia JA.

Fuente: Vaccine. 2004 Dec 21;23(6):819-26.

En: PubMed

468
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Lista de resultados combinados | PubMed (1876) | EBM Reviews, Cochrane Database (0) | Evidence Based Medicine Review - EBM (82) | Medline - OVID (2104) | OVID - Journals@Ovid (12) | GeoRef (0) | American Chemical Society - ACS (0) | Google Scholar (4650) | FindArticles (Free Articles Only) (0) | MEDLINE with Full Text (10)

☐ 21Registro Completo  

Título: DNA and low titer, helper-free, recombinant AAV prime-boost vaccination for cytomegalovirus induces an immune response to CMV-pp65 and CMV-IE1 in transgenic HLA A*0201 mice.

Autores: (eng)

En: MEDLINE with Full Text

☐ 22Registro Completo  

Título: Transient production of recombinant proteins by Chinese hamster ovary cells using polyethyleneimine/DNA complexes in combination with microtubule disrupting anti-mitotic agents.

Autores: (eng)

En: MEDLINE with Full Text

☐ 23Registro Completo  

Título: Intracellular viral processing, not single-stranded DNA accumulation, is crucial for recombinant adeno-associated virus transduction.

Autores: (eng)

En: MEDLINE with Full Text

☐ 24Registro Completo  

Título: Combined vaccine regimen based on parenteral priming with a DNA vaccine and administration of an oral booster consisting of a recombinant Salmonella enterica serovar Typhimurium vaccine strain for immunization against infection with human-derived enterotoxigenic Escherichia coli strains.

Autores: (eng)

En: MEDLINE with Full Text

☐ 25Registro Completo  

Título: Recombinant DNA vaccine encoding multiple domains related to inhibition of neurite outgrowth: a potential strategy for axonal regeneration.

Autores: (eng)

En: MEDLINE with Full Text

☐ 26Registro Completo  

Título: Binding of MCF-7 cell mitochondrial proteins and recombinant human estrogen receptors alpha and beta to human mitochondrial DNA estrogen response elements.

Autores: (eng)

En: MEDLINE with Full Text

☐ 27Registro Completo  

Título: Recombinant protein and DNA vaccines derived from hsp60 Trichophyton mentagrophytes control the clinical course of trichophytosis in bovine species and guinea-pigs.

Autores: (eng)

En: MEDLINE with Full Text

☐ 28Registro Completo  

Título: Protection of pigs against Taenia solium cysticercosis using recombinant antigen or in combination with DNA vaccine.

Autores: (eng)

En: MEDLINE with Full Text

☐ 29Registro Completo  

Título: Shuffled: a software suite that assists the analysis of recombinant products resulting from DNA shuffling.

469 27

En: **MEDLINE with Full Text**

☐ 30

Registro Completo  LinkSolver

Título: **A consecutive priming-boosting vaccination of mice with simian immunodeficiency virus (SIV) gag/pol DNA and recombinant vaccinia virus strain DIs elicits effective anti-SIV immunity.**

Autores: (eng

En: **MEDLINE with Full Text**

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Teraya, Tsurumi-ku, Yokohama-shi, Kanagawa-ken
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(54) Title: COMBINATIONS OF ANTIBODIES SELECTIVE FOR A TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-
INDUCING LIGAND RECEPTOR AND OTHER THERAPEUTIC AGENTS

(57) Abstract: An antibody of the invention interacts with human DR5 or with human DR4 to produce agonistic or antagonistic effects downstream of the receptor including inhibition of cell proliferation and apoptosis. Methods and uses for the antibodies, optionally in combination with various therapeutic agents, are detailed, including treatment of apoptosis-related disease and treatment of dysregulated cell growth.

What is claimed is:

1. A method of selectively inducing apoptosis in target cells expressing DR5, comprising the steps of (a) contacting the target cells with a therapeutic quantity of an antibody that specifically binds a TRAIL receptor DR5, wherein said antibody, in its soluble form, has *in vivo* and *in vitro* apoptosis-inducing activity in target cells expressing DR5 and (b) contacting the target cells with a therapeutic quantity of one or more therapeutic agents.
2. The method of claim 1, wherein at least one contacting step is performed *in vivo*.
3. The method of claim 1, wherein a least one contacting step is performed *in vitro*.
4. The method of claim 1, wherein the therapeutic agent or agents are chemotherapeutic agents.
5. The method of claim 4, wherein the chemotherapeutic agent or agents are selected from the group consisting of bleomycin, carboplatin, chlorambucil, cisplatin, colchicine, cyclophosphamide, daunorubicin, actinomycin, diethylstilbestrol, doxorubicin, etoposide, 5-fluorouracil, floxuridine, melphalan, methotrexate, mitomycin, 6-mercaptopurine, teniposide, 6-thioguanine, vincristine and vinblastine.
6. The method of claim 4, wherein the chemotherapeutic agent or agents are selected from the group consisting of leflunomide, dactinomycin, tamoxifen, interferon α -2b, glutamic acid, plicamycin, mercaptopurine, 6-thioguanine, carmustine, BCNU, lomustine, CCNU, cytosine araboside, estramustine, hydroxyurea, procarbazine, busulfan, medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, testolactone, melphalan, chlorambucil, mechlorethamine, thiotepa,

bethamethasone sodium phosphate, dicarbazine, asparaginase, mitotane, vincristine sulfate, and vinblastine sulfate.

7. The method of claim 4, wherein the chemotherapeutic agents are cyclophosphamide, doxorubicin, vincristine, and prednisone or a subset thereof.
8. The method of claim 7, further comprising administering rituximab.
9. The method of claim 1 wherein the therapeutic agent or agents are members of the TNF family.
10. The method of claim 9, wherein the member or members of the TNF family are CD40 ligands, or fragments or derivatives thereof.
11. The method of claim 9, wherein the member or members of the TNF family are Fas ligands, or a fragments or derivatives thereof.
12. The method of claim 1, wherein the therapeutic agent or agents are anti-inflammatory agents.
13. The method of claim 12, wherein the anti-inflammatory agent or agents are non-steroidal anti-inflammatory agents.
14. The method of claim 13, wherein non-steroidal anti-inflammatory agents are COX-1 inhibitors or COX-2 inhibitors.
15. The method of claim 12, wherein the anti-inflammatory agent or agents are steroidal anti-inflammatory agents.
16. The method of claim 1, wherein the therapeutic agent or agents are antiviral agents.
17. The method of claim 1, wherein the therapeutic agent or agents are anti-retroviral agents.
18. The method of claim 1, wherein the therapeutic agent or agents are anti-opportunistic agents.

19. The method of claim 1, wherein the therapeutic agent or agents are antibiotics.
20. The method of claim 1, wherein the therapeutic agent or agents are immunosuppressive agents.
21. The method of claim 1, wherein the therapeutic agent or agents are immunoglobulin preparations.
22. The method of claim 1, wherein the therapeutic agent or agents are antimalarial agents.
23. The method of claim 1, wherein the therapeutic agent or agents are disease modifying anti-rheumatic drugs.
24. The method of claim 1, wherein the therapeutic agent or agents are cytokines.
25. The method of claim 1, wherein the therapeutic agent or agents are chemokines.
26. The method of claim 1, wherein the therapeutic agent or agents are growth factors.
27. The method of claim 1, wherein the therapeutic agent is an apoptosis-inducing compound.
28. The method of claim 27, wherein the apoptosis-inducing compound is, bisindolylmaleimide VIII (BisVIII), SN-50 or LY294002.
29. The method of claim 1, wherein the therapeutic agent is a second antibody that promotes apoptosis or blocks proliferation of the target cells.
30. The method of claim 29, wherein the second antibody is selected from the group consisting of a DR4 antibody, a TNF antibody, a B7 antibody, a CD40 ligand antibody, a CD40 antibody, a CD20 antibody, and a Fas antibody.

31. The method of claim 1, wherein the therapeutic agent or agents are selected from the group consisting of chemotherapeutic agents, members of the TNF family, anti-inflammatory agents, anti-viral, anti-retroviral, anti-opportunistic agents, antibiotics, immunosuppressive agents, immunoglobulins, antimalarial agents, anti-rheumatoid arthritis agents, cytokines, chemokines, growth factors, and second antibody that promotes apoptosis or blocks proliferation of the target cells.
32. The method of claim 1, wherein the target cell is an abnormally proliferating synovial cell.
33. The method of claim 32, wherein the synovial cell is a rheumatoid arthritis synovial cell.
34. The method of claim 1, wherein the target cell is an activated immune cell.
35. The method of claim 34, wherein the activated immune cell is an activated lymphocyte.
36. The method of claim 1, wherein the target cell is a neutrophil.
37. The method of claim 1, wherein the target cell is a virally infected cell.
38. The method of claim 1, further comprising irradiating the target cells.
39. A method of inhibiting proliferation of target cells expressing DR5, comprising the steps of (a) contacting the target cells with a therapeutic quantity of an antibody that specifically binds a TRAIL receptor DR5, wherein said antibody, in its soluble form, has *in vivo* and *in vitro* apoptosis-inducing activity in cells expressing DR5 and (b) contacting the target cells with a therapeutic quantity of one or more therapeutic agents.
40. The method of claim 39, wherein at least one contacting step is performed *in vivo*.
41. The method of claim 39, wherein a least one contacting step is performed *in vitro*.

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42. The method of claim 39, wherein the therapeutic agent or agents are chemotherapeutic agents.
43. The method of claim 42, wherein the chemotherapeutic agent or agents are selected from the group consisting of bleomycin, carboplatin, chlorambucil, cisplatin, colchicine, cyclophosphamide, daunorubicin, actinomycin, diethylstilbestrol, doxorubicin, etoposide, 5-fluorouracil, floxuridine, melphalan, methotrexate, mitomycin, 6-mercaptopurine, teniposide, 6-thioguanine, vincristine and vinblastine.
44. The method of claim 42, wherein the chemotherapeutic agent or agents are selected from the group consisting of dactinomycin, tamoxifen, interferon α -2b, glutamic acid, plicamycin, mercaptopurine, 6-thioguanine, carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, estramustine, hydroxyurea, procarbazine, busulfan, medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, testolactone, mephalen, chlorambucil, mechlorethamine, thiotepa, bethamethasone sodium phosphate, dicarbazine, asparaginase, mitotane, vincristine sulfate, and vinblastine sulfate.
45. The method of claim 42, wherein the chemotherapeutic agents are cyclophosphamide, doxorubicin, vincristine, and prednisone or a subset thereof.
46. The method of claim 45, further comprising administering rituximab.
47. The method of claim 39, wherein the therapeutic agent or agents are members of the TNF family.
48. The method of claim 47, wherein the member or members of the TNF family are CD40 ligands, or fragments or derivatives thereof.

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49. The method of claim 47, wherein the member or members of the TNF family are Fas ligands, or a fragments or derivatives thereof.
50. The method of claim 39, wherein the therapeutic agent or agents are anti-inflammatory agents.
51. The method of claim 50, wherein the anti-inflammatory agent or agents are non-steroidal anti-inflammatory agents.
52. The method of claim 51, wherein non-steroidal anti-inflammatory agents are COX-1 inhibitors or COX-2 inhibitors.
53. The method of claim 50, wherein the anti-inflammatory agent or agents are steroidal anti-inflammatory agents.
54. The method of claim 39, wherein the therapeutic agent or agents are antiviral agents.
55. The method of claim 39, wherein the therapeutic agent or agents are anti-retroviral agents.
56. The method of claim 39, wherein the therapeutic agent or agents are anti-opportunistic agents.
57. The method of claim 39, wherein the therapeutic agent or agents are antibiotics.
58. The method of claim 39, wherein the therapeutic agent or agents are immunosuppressive agents.
59. The method of claim 39, wherein the therapeutic agent or agents are immunoglobulin preparations.
60. The method of claim 39, wherein the therapeutic agent or agents are antimalarial agents.
61. The method of claim 39, wherein the therapeutic agent or agents are anti-rheumatoid arthritis agents.

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62. The method of claim 39, wherein the therapeutic agent or agents are cytokines
63. The method of claim 39, wherein the therapeutic agent or agents are chemokines.
64. The method of claim 39, wherein the therapeutic agent or agents are growth factors.
65. The method of claim 39, wherein the therapeutic agent is a second antibody that promotes apoptosis or blocks proliferation of the target cells.
66. The method of claim 65, wherein the second antibody is selected from the group consisting of a DR4 antibody, a TNF antibody, a B7 antibody, a CD40 ligand antibody, a CD40 antibody, a CD20 antibody, and a Fas antibody.
67. The method of claim 1, wherein the therapeutic agent is an apoptosis-inducing compound.
68. The method of claim 67, wherein the apoptosis-inducing compound is, bisindolylmaleimide VIII (BisVIII), SN-50 or LY294002.
69. The method of claim 39, wherein the therapeutic agent or agents are selected from the group consisting of chemotherapeutic agents, members of the TNF family, anti-inflammatory agents, anti-viral, anti-retroviral, anti-opportunistic agents, antibiotics, immunosuppressive agents, immunoglobulins, antimalarial agents, anti-rheumatoid arthritis agents, cytokines, chemokines, growth factors, and second antibody that promotes apoptosis or blocks proliferation of the target cells.
70. The method of claim 39, wherein the target cell is an abnormally proliferating synovial cell.
71. The method of claim 70, wherein the synovial cell is a rheumatoid arthritis synovial cell.
72. The method of claim 39, wherein the target cell is an activated immune cell.

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73. The method of claim 39, wherein the target cell is an activated lymphocyte.
74. The method of claim 39, wherein the target cell is a neutrophil.
75. The method of claim 39, wherein the target cell is a virally infected cell.
76. The method of claim 39, further comprising irradiating the target cells.
77. A method of treating a subject having an inflammatory or autoimmune disease, comprising (a) administering to the subject a therapeutic amount of an antibody that specifically binds a TRAIL receptor DR5, wherein said antibody, in its soluble form, has *in vivo* and *in vitro* apoptosis-inducing activity in target cells expressing DR5 and (b) administering to the subject a therapeutic quantity of a therapeutic agent.
78. The method of claim 77, wherein the therapeutic agent or agents are chemotherapeutic agents.
79. The method of claim 78, wherein the chemotherapeutic agent or agents are selected from the group consisting of bleomycin, carboplatin, chlorambucil, cisplatin, colchicine, cyclophosphamide, daunorubicin, actinomycin, diethylstilbestrol, doxorubicin, etoposide, 5-fluorouracil, floxuridine, melphalan, methotrexate, mitomycin, 6-mercaptopurine, teniposide, 6-thioguanine, vincristine and vinblastine.
80. The method of claim 78, wherein the chemotherapeutic agent or agents are selected from the group consisting of dactinomycin, tamoxifen, interferon α -2b, glutamic acid, plicamycin, mercaptopurine, 6-thioguanine, carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, estramustine, hydroxyurea, procarbazine, busulfan, medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, testolactone, melphalan, chlorambucil, mechlorethamine, thiotepa, bethamethasone sodium phosphate,

dicarbazine, asparaginase, mitotane, vincristine sulfate, and vinblastine sulfate.

81. The method of claim 78, wherein the chemotherapeutic agents are cyclophosphamide, doxorubicin, vincristine, and predisone or a subset thereof.
82. The method of claim 81, further comprising administering rituximab.
83. The method of claim 77, wherein the therapeutic agent or agents are members of the TNF family.
84. The method of claim 83, wherein the member or members of the TNF family are CD40 ligands, or fragments or derivatives thereof.
85. The method of claim 83, wherein the member or members of the TNF family are Fas ligands, or a fragments or derivatives thereof.
86. The method of claim 77, wherein the therapeutic agent or agents are anti-inflammatory agents.
87. The method of claim 86, wherein the anti-inflammatory agent or agents are non-steroidal anti-inflammatory agents.
88. The method of claim 87, wherein non-steroidal anti-inflammatory agents are COX-1 inhibitors or COX-2 inhibitors.
89. The method of claim 86, wherein the anti-inflammatory agent or agents are steroidal anti-inflammatory agents.
90. The method of claim 77, wherein the therapeutic agent or agents are antiviral agents.
91. The method of claim 77, wherein the therapeutic agent or agents are anti-retroviral agents.
92. The method of claim 77, wherein the therapeutic agent or agents are anti-opportunistic agents.

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93. The method of claim 77, wherein the therapeutic agent or agents are antibiotics.
94. The method of claim 77, wherein the therapeutic agent or agents are immunosuppressive agents.
95. The method of claim 77, wherein the therapeutic agent or agents are immunoglobulin preparations.
96. The method of claim 77, wherein the therapeutic agent or agents are antimalarial agents.
97. The method of claim 77, wherein the therapeutic agent or agents are anti-rheumatoid arthritis agents.
98. The method of claim 77, wherein the therapeutic agent or agents are cytokines
99. The method of claim 77, wherein the therapeutic agent or agents are chemokines.
100. The method of claim 77, wherein the therapeutic agent or agents are growth factors.
101. The method of claim 77, wherein the therapeutic agent is a second antibody that promotes apoptosis or blocks proliferation of the target cells.
102. The method of claim 101, wherein the second antibody is selected from the group consisting of a DR4 antibody, a TNF antibody, a B7 antibody, a CD40 ligand antibody, a CD40 antibody, a CD20 antibody, and a Fas antibody.
103. The method of claim 1, wherein the therapeutic agent is an apoptosis-inducing compound.
104. The method of claim 103, wherein the apoptosis-inducing compound is, bisindolylmaleimide VIII (BisVIII), SN-50 or LY294002.

105. The method of claim 77, wherein the therapeutic agent or agents are selected from the group consisting of chemotherapeutic agents, members of the TNF family, anti-inflammatory agents, anti-viral, anti-retroviral, anti-opportunistic agents, antibiotics, immunosuppressive agents, immunoglobulins, antimalarial agents, anti-rheumatoid arthritis agents, cytokines, chemokines, growth factors, and second antibody that promotes apoptosis or blocks proliferation of the target cells.
106. The method of claim 77, wherein the inflammatory or autoimmune disease is selected from the group consisting of systemic lupus erythematosus, Hashimoto's disease, rheumatoid arthritis, graft-versus-host disease, Sjögren's syndrome, pernicious anemia, Addison disease, scleroderma, Goodpasture's syndrome, Crohn's disease, autoimmune hemolytic anemia, sterility, myasthenia gravis, multiple sclerosis, Basedow's disease, thrombotic thrombocytopenia, thrombopenia purpura, insulin-dependent diabetes mellitus, allergy; asthma, atopic disease; arteriosclerosis; myocarditis; cardiomyopathy; glomerular nephritis; hypoplastic anemia.
107. The method of claim 77, further comprising administering to the subject radiation therapy.
108. A composition comprising (a) an antibody that specifically binds a TRAIL receptor DR5, wherein said antibody, in its soluble form, has *in vivo* and *in vitro* apoptosis-inducing activity in target cells expressing DR5 and (b) one or more therapeutic agents.
109. The composition of claim 108, wherein the therapeutic agent or agents are selected from the group consisting of chemotherapeutic agents, members of the TNF family, anti-inflammatory agents, anti-viral, anti-retroviral, anti-opportunistic agents, antibiotics, immunosuppressive agents, immunoglobulins, antimalarial agents, disease modifying anti-rheumatic

drug, cytokines, chemokines, growth factors, and second antibodies that promotes apoptosis or blocks proliferation of the target cells.

110. The composition of claim 108, further comprising a pharmaceutically acceptable carrier.

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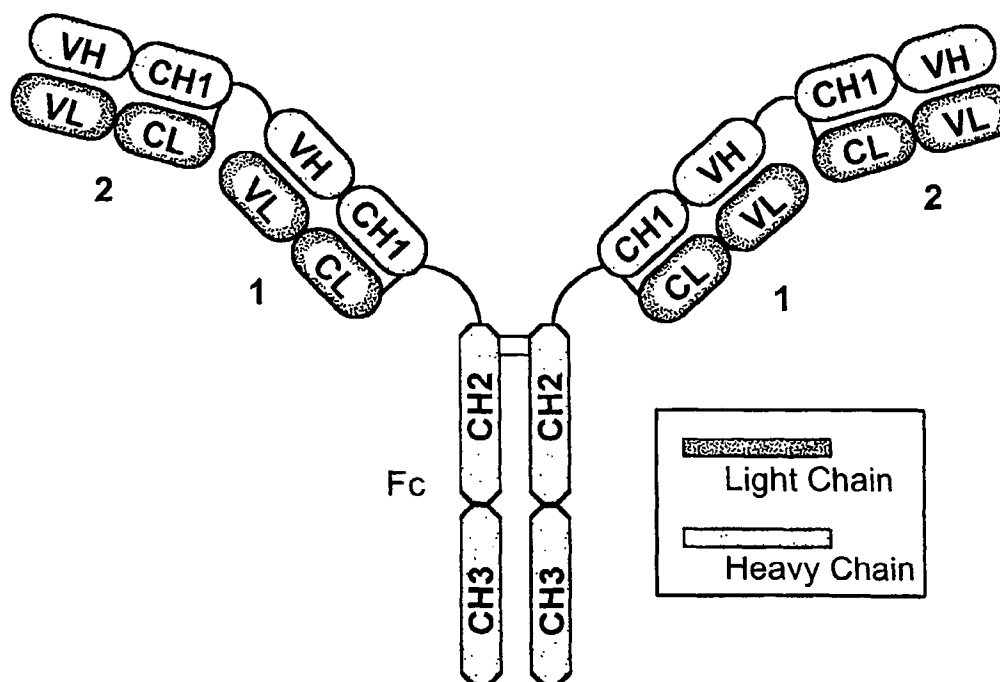
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(54) Title: MULTIVALENT ANTIBODIES AND USES THEREFOR



WO 01/77342 A1

(57) Abstract: The present application describes engineered antibodies, with three or more functional antigen binding sites, and uses, such as therapeutic applications, for such engineered antibodies.

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WHAT IS CLAIMED IS:

1. An isolated antibody comprising an Fc region and three or more antigen binding sites amino-terminal to the Fc region.
- 5 2. The antibody of claim 1 comprising four antigen binding sites.
3. The antibody of claim 1 comprising five or more antigen binding sites.
- 10 4. The antibody of claim 1 comprising a polypeptide chain, wherein the polypeptide chain comprises two or more variable domains.
5. The antibody of claim 4 wherein the polypeptide chain comprises $VD1-(X1)_n-VD2-(X2)_n-Fc$, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1.
- 15 6. The antibody of claim 5 comprising two or more polypeptide chains, each comprising $VD1-(X1)_n-VD2-(X2)_n-Fc$.
- 20 7. The antibody of claim 1 comprising at least one polypeptide chain with the formula:
(a) VH-CH1-flexible linker-VH-CH1-Fc region chain; or
(b) VH-CH1-VH-CH1-Fc region chain.
8. The antibody of claim 1 comprising at least two light chain variable domain polypeptides.
- 25 9. The antibody of claim 8 wherein the light chain variable domain polypeptides further comprise a CL domain.
10. The antibody of claim 1 comprising a polypeptide chain, wherein the polypeptide chain comprises Fd-flexible linker-Fd.
- 30 11. The antibody of claim 10 wherein the flexible linker comprises a peptide selected from the group consisting of gly-ser, gly-ser-gly-ser (SEQ ID NO:10), ala-ser, and gly-gly-gly-ser (SEQ ID NO:11).
- 35 12. The antibody of claim 1 which is internalized faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind.
13. The antibody of claim 1 which is an agonist antibody.

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14. The antibody of claim 1 which induces apoptosis.
- 5 15. The antibody of claim 1 wherein the three or more antigen binding sites all bind the same antigen.
16. The antibody of claim 1 wherein the three or more antigen binding sites bind two or more different antigens.
- 10 17. The antibody of claim 1 which binds a cell surface protein expressed by tumor cells.
18. The antibody of claim 17 wherein the cell surface protein is selected from the group consisting of the Epidermal Growth Factor Receptor (EGFR), HER2 receptor, HER3 receptor, HER4 receptor and DcR3 receptor.
- 15 19. The antibody of claim 17 wherein the cell surface protein is HER2 receptor.
20. The antibody of claim 1 which binds a cell surface protein overexpressed by tumor cells.
- 20 21. The antibody of claim 1 which binds a receptor in the Tumor Necrosis Factor (TNF) receptor superfamily.
22. The antibody of claim 21 wherein the TNF receptor is an Apo2L receptor.
- 25 23. The antibody of claim 22 wherein the Apo2L receptor is selected from the group consisting of DR4, DR5, DcR1 and DcR2.
24. The antibody of claim 22 wherein the Apo2L receptor is DR4 or DR5.
- 30 25. The antibody of claim 21 which is an agonist antibody.
26. The antibody of claim 21 which induces apoptosis.
27. The antibody of claim 1 which binds a B cell surface antigen.
- 35 28. The antibody of claim 27 wherein the B cell surface antigen is selected from the group consisting of CD19, CD20, CD22 and CD40.
29. The antibody of claim 27 wherein the B cell surface antigen is CD20.

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30. An immunoconjugate comprising the antibody of claim 1 conjugated with a cytotoxic agent.
- 5 31. The immunoconjugate of claim 30 wherein the cytotoxic agent is active in killing cells once internalized.
32. The immunoconjugate of claim 30 wherein the cytotoxic agent is selected from the group consisting of a radioactive isotope, a maytansinoid and a calicheamicin.
- 10 33. An isolated antibody comprising three or more antigen binding sites, wherein the antibody is capable of binding a receptor in the Tumor Necrosis Factor (TNF) receptor superfamily.
34. The antibody of claim 33 which is not a native sequence IgM or IgA antibody.
- 15 35. The antibody of claim 33 which has only one Fc region or lacks an Fc region.
36. The antibody of claim 33 which comprises a polypeptide chain, wherein the polypeptide chain comprises two or more variable domains.
- 20 37. The antibody of claim 33 which comprises four antigen binding sites each capable of binding the TNF receptor.
38. The antibody of claim 33 wherein the TNF receptor is an Apo2L receptor.
- 25 39. The antibody of claim 38 wherein the Apo2L receptor is selected from the group consisting of DR4, DR5, DcR1 and DcR2.
40. The antibody of claim 38 wherein the Apo2L receptor is DR4 or DR5.
- 30 41. The antibody of claim 33 which is an agonist antibody.
42. The antibody of claim 33 which induces apoptosis.
43. An isolated antibody comprising at least three antigen binding sites, wherein the antibody is capable of binding an ErbB receptor.
- 35 44. The antibody of claim 43 which is not a native sequence IgM or IgA antibody.

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45. The antibody of claim 43 which has only one Fc region or lacks an Fc region.
46. The antibody of claim 43 which comprises a polypeptide chain, wherein the polypeptide chain comprises two or more variable domains.
- 5 47. The antibody of claim 43 which comprises four antigen binding sites each capable of binding the ErbB receptor.
- 10 48. The antibody of claim 43 wherein the ErbB receptor is selected from the group consisting of Epidermal Growth Factor Receptor (EGFR), HER2 receptor, HER3 receptor and HER4 receptor.
49. The antibody of claim 43 where the ErbB receptor is HER2 receptor.
- 15 50. An isolated antibody comprising at least three antigen binding sites, wherein the antibody is capable of binding a B cell surface antigen.
51. The antibody of claim 50 which is not a native sequence IgM or IgA antibody.
- 20 52. The antibody of claim 50 which has only one Fc region or lacks an Fc region.
53. The antibody of claim 50 which comprises a polypeptide chain, wherein the polypeptide chain comprises two or more variable domains.
- 25 54. The antibody of claim 50 which comprises four antigen binding sites each capable of the B cell surface antigen.
55. The antibody of claim 50 wherein the B cell surface antigen is selected from the group consisting of CD19, CD20, CD22 and CD40.
- 30 56. An isolated antibody comprising at least three antigen binding sites, wherein the antibody is capable of binding an antigen which is overexpressed by cancer cells.
57. A polypeptide chain comprising:
35 (a) VH-CH1-flexible linker-VH-CH1-dimerization domain; or
(b) VH-CH1-VH-CH1-dimerization domain.
58. An isolated antibody comprising the polypeptide chain of claim 57.

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59. The antibody of claim 58 further comprising two or more light chain variable domain polypeptides.
60. The antibody of claim 59 wherein the light chain variable domain polypeptides comprise VL-CL.
- 5 61. An isolated antibody comprising a dimerization domain and three or more antigen binding sites amino-terminal thereto
62. The antibody of claim 61 wherein the dimerization domain is selected from the group consisting of a hinge region, an Fc region, a CH3 domain, and a CH4 domain.
- 10 63. The antibody of claim 62 wherein the dimerization domain is a hinge region.
64. The antibody of claim 63 wherein the dimerization domain further comprises a leucine zipper.
- 15 65. The antibody of claim 63 comprising a polypeptide chain comprising the formula:
(a) VH-CH1-flexible linker-VH-CH1-hinge region; or
(b) VH-CH1-VH-CH1-hinge region.
- 20 66. A polypeptide chain comprising three or more heavy chain or light chain variable domains, wherein each of the variable domains is able to combine with three or more light chain or heavy chain variable domain polypeptides to form three or more antigen binding sites, each directed against the same antigen.
- 25 67. The polypeptide chain of claim 66 which comprises three heavy chain variable domains which are able to combine with three light chain variable domain polypeptides to form three antigen binding sites directed against the same antigen.
- 30 68. The polypeptide chain of claim 66 which comprises four heavy chain variable domains which are able to combine with four light chain variable domain polypeptides to form four antigen binding sites directed against the same antigen.
69. The polypeptide chain of claim 66 wherein the antigen is a receptor in the Tumor Necrosis Factor (TNF) receptor superfamily.
- 35 70. The polypeptide chain of claim 66 wherein the antigen is a B cell surface antigen.
71. The polypeptide chain of claim 66 wherein the antigen is an ErbB receptor.

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72. The polypeptide chain of claim 66 wherein the antigen is a cell surface protein expressed by tumor cells.
73. A polypeptide chain comprising the formula:
- 5 (a) VL-CL-flexible linker-VL-CL-flexible linker-VL-CL;
(b) VH-CH1-flexible linker-VH-CH1-flexible linker-VH-CH1;
(c) (VL-CL)_n, wherein n is three or more; or
(d) (VH-CH1)_n, wherein n is three or more.
- 10 74. The polypeptide chain of claim 67 comprising the formula:
- (a) VH-CH1-flexible linker-VH-CH1-flexible linker-VH-CH1;
(b) VH-CH1-flexible linker-VH-CH1-flexible linker-VH-CH1-flexible linker-VH-CH1; or
(c) (VH-CH1)_n, wherein n is three or four.
- 15 75. An isolated antibody comprising the polypeptide chain of claim 66.
76. The isolated antibody of claim 75 further comprising the three or more light chain or heavy chain variable domain polypeptides.
- 20 77. The isolated antibody of claim 76 comprising three or more light chain variable domain polypeptides, each comprising VL-CL.
78. The isolated antibody of claim 77 comprising four light chain variable domain polypeptides, each comprising VL-CL.
- 25 79. A polypeptide chain comprising:
- (a) VL-CL-flexible linker-VL-CL-dimerization domain;
(b) VL-CL-VL-CL-dimerization domain.
- 30 80. An immunoconjugate comprising the antibody of claim 75 conjugated with a cytotoxic agent.
81. Isolated nucleic acid encoding the antibody of claim 1, the polypeptide chain of claim 57 or the polypeptide chain of claim 66.
- 35 82. A vector comprising the nucleic acid of claim 81.
83. A host cell comprising the nucleic acid of claim 81.

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84. A process of producing an antibody or polypeptide chain comprising culturing the host cell of claim 83 so that the nucleic acid is expressed.
- 5 85. The process of claim 84 further comprising recovering the antibody or polypeptide chain from the host cell culture.
86. The process of claim 85 wherein the antibody or polypeptide chain is recovered from the host cell culture medium.
- 10 87. A method for treating a disorder in a mammal comprising administering to the mammal a therapeutically effective amount of the antibody of claim 1.
88. The method of claim 87 wherein the disorder is cancer.
- 15 89. The method of claim 87 further comprising administering a therapeutically effective amount of a cytotoxic agent to the mammal.
90. A method of inducing apoptosis of a cancer cell comprising exposing the cell to the antibody of claim 33.
- 20 91. A method of killing a B cell comprising exposing the B cell to the antibody of claim 50.
92. A method of killing a cell which expresses an ErbB receptor comprising exposing the cell to the antibody of claim 43.
- 25 93. The method of claim 92 wherein the cell is a cancer cell which overexpresses an ErbB receptor.

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(54) Title: ANTIBODIES TO DEATH RECEPTOR 4 (DR4) AND USES THEREOF (57) Abstract Death Receptor 4 (DR4) antibodies are provided. The DR4 antibodies may be included in pharmaceutical compositions, articles of manufacture, or kits. Methods of treatment and diagnosis using the DR4 antibodies are also provided.			

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What is claimed is:

1. An antibody which specifically binds to DR4.
- 5 2. The antibody of Claim 1 which is an agonist antibody.
3. The antibody of Claim 1 which is a blocking antibody.
4. The antibody of Claim 1 which is a monoclonal antibody.
- 10 5. The antibody of claim 1 which is a chimeric antibody.
6. A hybridoma cell line which produces the antibody of Claim 4.
- 15 7. The antibody of Claim 4 having the biological characteristics of (1) the monoclonal antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-12454 or (2) the monoclonal antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-12455.
- 20 8. The antibody of Claim 4 wherein the antibody binds to the same epitope as (1) the epitope to which the monoclonal antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-12454 binds or (2) the epitope to which the monoclonal antibody produced by the hybridoma cell line deposited under the American Type Culture Collection Accession Number ATCC HB-12455 binds.
- 25 9. The hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-12454.
- 30 10. The hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-12455.
- 35 11. The monoclonal antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-12454.

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12. The monoclonal antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-12455.
- 5 13. An isolated nucleic acid encoding the DR4 antibody of Claim 1.
14. A composition comprising the antibody of Claim 1 and a carrier.
15. The composition of claim 14 wherein said carrier is a pharmaceutically acceptable
10 carrier.
16. A method of inducing apoptosis in mammalian cells comprising exposing mammalian cells to an effective amount of a DR4 agonist antibody.
- 15 17. An article of manufacture, comprising a container and a composition contained within said container, wherein the composition includes a DR4 antibody.
18. The article of manufacture of claim 17 further comprising instructions for using the DR4 antibody *in vivo* or *ex vivo*.
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19. A dimeric molecule comprising a DR4 antibody linked to a heterologous immunoglobulin.