

①②

**EUROPEAN PATENT APPLICATION**

②① Application number: 87302620.7

⑤① Int. Cl.⁴: **C 12 N 15/00, C 07 K 15/06,**  
**C 12 P 21/02**

②② Date of filing: 26.03.87

③④ Priority: 27.03.86 GB 8607679

⑦① Applicant: **Winter, Gregory Paul, 64 Cavendish Avenue, Cambridge (GB)**

④③ Date of publication of application: 30.09.87  
Bulletin 87/40

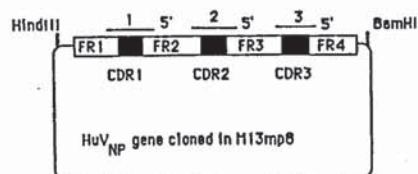
⑦② Inventor: **Winter, Gregory Paul, 64 Cavendish Avenue, Cambridge (GB)**

⑥④ Designated Contracting States: **AT BE CH DE ES FR GB GR IT LI LU NL SE**

⑦④ Representative: **Votier, Sidney David et al, CARPMAELS & RANSFORD 43, Bloomsbury Square, London WC1A 2RA (GB)**

⑥⑤ **Recombinant antibodies and methods for their production.**

⑥⑦ An altered antibody is produced by replacing the complementarity determining regions (CDRs) of a variable region of an immunoglobulin (Ig) with the CDRs from an Ig of different specificity, using recombinant DNA techniques. The gene coding sequences for producing the altered antibody may be produced by site-directed mutagenesis using long oligonucleotides.



D1.3 CDR1 oligonucleotide  
5' CTG,TCT,CAC,CCA,GGT,TAC,ACC,ATA,GCC,GCT,GAA,GGT,GCT  
FR2 D1.3 CDR1 FR1

D1.3 CDR2 oligonucleotide  
5' CAT,TGT,CAC,TCT,GGG,TTT,GAG,AGC,TGA,ATT,ATA,GTC,TGT,  
FR3 D1.3 CDR2  
GTT,TCC,ATC,ACC,CCA,AAT,CAT,TCC,AAT,CCA,CTC  
D1.3 CDR2 FR2

D1.3 CDR3 oligonucleotide  
5' GCC,TTG,ACC,CCA,ATA,GTC,AA6,CCT,ATA,ATC,TCT,CTC,TCT,  
FR4 D1.3 CDR3  
TGC,ACA,ATA  
FR3

**EP 0 239 400 A2**

TITLE MODIFIED  
see front page

-1-

RECOMBINANT DNA PRODUCT AND METHODS

The present invention relates to altered antibodies in which at least parts of the complementarity determining regions (CDRs) in the light or heavy chain variable domains of the antibody have been replaced by analogous parts of CDRs from an antibody of different specificity. The present invention also relates to methods for the production of such altered antibodies.

Natural antibodies, or immunoglobulins, comprise two heavy chains linked together by disulphide bonds and two light chains, one light chain being linked to each of the heavy chains by disulphide bonds. The general structure of an antibody of class IgG (i.e. an immunoglobulin (Ig) of class gamma (G)) is shown schematically in Figure 1 of the accompanying drawings.

Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end, the variable domain being aligned with the variable domain of the heavy chain and the constant domain being aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to the antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises four framework

regions, whose sequences are relatively conserved, connected by three hypervariable or complementarity determining regions (CDRs) (see Kabat, E.A., Wu, T.T., Bilofsky, H., Reid-Miller, M. and Perry, H., in "Sequences of Proteins of Immunological Interest", US Dept. Health and Human Services 1983). The four framework regions largely adopt a  $\beta$ -sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the  $\beta$ -sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site.

For a more detailed account of the structure of variable domains, reference may be made to: Poljak, R.J., Amzel, L.M., Avey, H.P., Chen, B.L., Phizackerly, R.P. and Saul, F., PNAS USA, 70, 3305-3310, 1973; Segal, D.M., Padlan, E.A., Cohen, G.H., Rudikoff, S., Potter, M. and Davies, D.R., PNAS USA, 71, 4298-4302, 1974; and Marquart, M., Deisenhofer, J., Huber, R. and Palm, W., J. Mol. Biol., 141, 369-391, 1980.

In recent years advances in molecular biology based on recombinant DNA techniques have provided processes for the production of a wide range of heterologous polypeptides by transformation of host cells with heterologous DNA sequences which code for the production of the desired products.

EP-A-0 088 994 (Schering Corporation) proposes the construction of recombinant DNA vectors comprising a ds DNA sequence which codes for a variable domain of a light or a heavy chain of an Ig specific for a

predetermined ligand. The ds DNA sequence is provided with initiation and termination codons at its 5'- and 3'- termini respectively, but lacks any nucleotides coding for amino acids superfluous to the variable domain. The ds DNA sequence is used to transform bacterial cells. The application does not contemplate variations in the sequence of the variable domain.

EP-A-1 102 634 (Takeda Chemical Industries Limited) describes the cloning and expression in bacterial host organisms of genes coding for the whole or a part of human IgE heavy chain polypeptide, but does not contemplate variations in the sequence of the polypeptide.

EP-A-0 125 023 (Genentech Inc.) proposes the use of recombinant DNA techniques in bacterial cells to produce Ig's which are analogous to those normally found in vertebrate systems and to take advantage of the gene modification techniques proposed therein to construct chimeric Igs or other modified forms of Ig.

The term 'chimeric antibody' is used to describe a protein comprising at least the antigen binding portion of an immunoglobulin molecule (Ig) attached by peptide linkage to at least part of another protein.

It is believed that the proposals set out in the above Genentech application did not lead to the expression of any significant quantities of Ig polypeptide chains, nor to the production of Ig activity, nor to the secretion and assembly of the chains into the desired chimeric Igs.

The production of monoclonal antibodies was first disclosed by Kohler and Milstein (Kohler, G. and Milstein, C., *Nature*, 256, 495-497, 1975). Such monoclonal antibodies have found widespread use not only as diagnostic reagents (see, for example, 'Immunology for the 80s, Eds. Voller, A., Bartlett, A., and Bidwell, D., MTP Press, Lancaster, 1981) but also in therapy (see, for example, Ritz, J. and Schlossman, S.F., *Blood*, 59, 1-11, 1982).

The recent emergence of techniques allowing the stable introduction of Ig gene DNA into myeloma cells (see, for example, Oi, V.T., Morrison, S.L., Herzenberg, L.A. and Berg, P., *PNAS USA*, 80, 825-829, 1983; Neuberger, M.S., *EMBO J.*, 2, 1373-1378, 1983; and Ochi, T., Hawley, R.G., Hawley, T., Schulman, M.J., Traunecker, A., Kohler, G. and Hozumi, N., *PNAS USA*, 80, 6351-6355, 1983), has opened up the possibility of using in vitro mutagenesis and DNA transfection to construct recombinant Igs possessing novel properties.

However, it is known that the function of an Ig molecule is dependent on its three dimensional structure, which in turn is dependent on its primary amino acid sequence. Thus, changing the amino acid sequence of an Ig may adversely affect its activity. Moreover, a change in the DNA sequence coding for the Ig may affect the ability of the cell containing the DNA sequence to express, secrete or assemble the Ig.

It is therefore not at all clear that it will be possible to produce functional altered antibodies by recombinant DNA techniques.

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

## E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.