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RESEARCH ARTICLE

RITUXIMAB: A RECOMBINANT CHIMERIC MONOCLONAL ANTI CD20 ANTIBODY

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ABSTRACT

Rituximab is a recombinant therapeutic chimeric mouse/human monoclonal antibody that binds to CD20; a transmembrane phosphorylated protein, located on pre-B and matures B-lymphocytes. It then recruits the body's natural defences to attack and kill the marked B-cells. It is primarily used to treat patients with relapsed low grade Non-Hodgkin's Lymphoma. The blood cancer therapy rituximab was the world's best-selling oncology drug in 2013, with nearly $8 billion in sales, according to industry data. This article describes the overview of rituximab including mechanism of action, indications and adverse reactions.

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INTRODUCTION

Monoclonal antibodies are produced by a clone of B cells and constitute a class of biotherapeutics which are a cornerstone of therapy for a wide range of disorders, from cancer to autoimmune disorders. Rituximab is one of the first monoclonal antibody developed by IDEC Pharmaceuticals under the name IDEC-C2B8. Based on its safety and effectiveness in clinical trials rituximab was approved by the U.S. Food and Drug Administration in 1997 to treat B-cell non-Hodgkin lymphomas resistant to other chemotherapy regimens (Maloney *et al.*, 1997; Scott, 1998). Rituximab is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes (Edwards *et al.*, 2004). Rituximab is a glycosylated IgG1 kappa immunoglobulin containing murine light and heavy chain variable region sequences (Fab domain) and human constant region sequences (Fc domain). Rituximab is composed of 1,328 amino acids and has an approximate molecular weight of 144.5 kD. Rituximab has a high binding affinity for the CD20 antigen of 5.2 to 11.0 nM. Over the years several biosimilars have been developed for rituximab in different parts of the world. Rituximab is available in the market with different trade names: Rituxan, MabThera, Reditux, Mabtas and Zytux.

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Rituximab is currently co-marketed by BiogenIdec and Genentech in the U.S., by Hoffmann-La Roche in Canada and the European Union, Chugai Pharmaceuticals, Zenyaku Kogyo in Japan and AryoGen in Iran. It is on the World Health Organization's list of the essential medicines, a list of the most important medications needed in a basic health system. Rituximab is produced by mammalian cell (Chinese Hamster Ovary) suspension culture in a nutrient medium. Rituximab is a sterile, clear, colourless, preservative-free liquid concentrate for intravenous administration and supplied at a concentration of 10 mg/mL in either 100 mg/10 mL or 500 mg/50 mL single-use vials. Rituximab is composed of two heavy chains of 451 amino acids and two light chains of 213 amino acids, with the following amino acid sequence (Anderson *et al.*, 1998).

Heavy chain amino acid sequence

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHW
VKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTAD
KSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNV
WGAGTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGC
LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Light chain amino acid sequence

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKP
GSSPKPWIYATSNLASGVPVRFSGSGSGTSYSLTISRVEA
EDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS
GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

PROCESS OF MANUFACTURING

The manufacture of rituximab begins with the selection of appropriate genetic sequence which has to be cloned into a suitable expression vector. Manufacturing also include thorough characterization of Chinese Hamster Ovary (CHO) master cell bank that contains the integrated gene coding for expression of rituximab. CHO cell are grown in suitable culture medium and purification of rituximab is based on chromatographic techniques such as affinity chromatography and anion exchange chromatography. The chromatography steps are designed to remove impurities such as host cell DNA, and host cell impurity proteins. Each step in the manufacturing process including scale-up, purification and formulation of the end product has a profound impact on the structure of the drug molecule (Lee *et al.*, 2012). Fundamental parameters, such as temperature, pH, agitation and the type of containers used, are a few basic factors that also influence the quality of the final product (Rathore and Rajan, 2008).

THERAPEUTIC INDICATIONS ANS USAGE

Rituximab is a prescription medicine indicated to treat:

- Non-Hodgkin's Lymphoma NHL), alone or with other chemotherapy medicines,
- Chronic lymphocytic leukemia (CLL) along with the chemotherapy medicines fludarabine and cyclophosphamide,
- Rheumatoid arthritis (RA) with another prescription medicine called methotrexate,
- Granulomatosis with Polyangiitis (GPA) and Microscopic Polyangiitis (MPA)

MECHANISM OF ACTION

Studies conducted on the mechanism of action of rituximab suggest that the drug interferes with the internal function of B-cells. Such studies indicate that rituximab can induce apoptosis (cell death) directly by interfering with CD20 calcium regulation and elevating the amount of calcium in the B-cells to abnormal levels. It may also interfere with various cell factors, causing outright cell death or inhibiting cell growth. Among these cell factors is Bcl-2, a member of a small family of closely related genes that can be divided into death-inhibiting genes, such as Bcl-2 and Bcl-xL, and death-promoting genes, such as Bax and Bad. The balance between death-promoting and death-inhibiting gene expression is critically important in both B- and T-cells, because these populations are regulated so that a person will, in the absence of infection, maintain a constant level of B- and T-cells despite the production and death of many of these cells each day. Typically, Bcl-2 and Bcl-xL are over-expressed in lymphomas, and rituximab appears to be able to interfere with their signaling pathways and reduce their expression. The over-expression of Bcl-xL may also play a role in making B-cell lymphomas more resistant to other types of chemotherapy; therefore adding rituximab to the therapeutic regimen appears to increase the effectiveness of the therapy.

There are also three main ways in which rituximab acts in concert with the body's own immune system cells once the rituximab antibody has entered the blood stream and attached to a B-cell. The mouse portion on one end of the rituximab antibody is the part that targets the CD20 antigen on the B-cell, while the other end of the antibody is human IgG, kappa type. When the mouse portion "locks" onto the CD20 "docking site" on the B-cell, the human IgG portion on the other end attracts or "recruits" the body's own immune system cells, also called effector cells, to respond. These effector cells include macrophages, neutrophils, and natural killer cells, and they attach to the rituximab antibody at a specific location called the Fc receptor site. This Fc receptor site is important and will be referred to later in the discussion on rituximab resistance. In this context, one can think of rituximab as a "bridge" that brings together the B-cell and the effector cell so that the B-cell can be destroyed.

One way or mechanism by which the B-cell is destroyed is through direct ingestion of the B-cell by the effector cell in a process called antibody-dependent phagocytosis. The effector cells in this scenario are usually macrophages, which are activated monocytes. Several studies have shown that this scenario occurs in laboratory cell lines, but other studies have suggested that this plays a relatively minor role in B-cell destruction in the body. A second and more important mechanism appears to be antibody-dependent cellular cytotoxicity (ADCC). In this case, the intact B-cell is not ingested or phagocytosed. Instead, certain effector cells (usually natural killer cells and possibly also macrophages) are triggered to release pore-forming proteins that penetrate the B-cell membrane and proteolytic enzymes that break up its structure and degrade its chromosomes. This process ultimately causes cell death through lysis (destruction of a cell by damage to its outer membrane).

A third important mechanism for B-cell destruction is referred to as complement-dependent cytotoxicity (CDC). Complement is a system of small proteins circulating in the blood that, when stimulated, form a series of enzymes which can directly attack the cell membrane or target the cell for destruction by phagocytosis. Its activity is said to "complement" the activity of antibodies, hence its name. Rituximab is capable of binding to complement proteins to initiate this response, in this case against the B-cells that it attaches to. It has been demonstrated that, when rituximab is infused into a patient, complement in the bloodstream is temporarily consumed or used up because of this process. Another theory suggests a fourth way that rituximab cooperates with the immune system. The suggestion is that B-cells coated with rituximab are capable of stimulating dendritic cells, which are special cells that are able to recognize antigens, present them to T-cells, and activate T-cells to attack the antigens, in this case the coated B-cells. This

may be one reason why rituximab appears to work for several months after actual treatment, even though blood and tissue levels of the drug may no longer be in the therapeutic range (Herms, 2008).

ADVERSE REACTIONS

Most common adverse reactions in clinical trials were

- NHL (≥ 25%): infusion reactions, fever, lymphopenia, chills, infection and asthenia
- CLL (≥ 25%): infusion reactions and neutropenia
- RA (≥ 10%): upper respiratory tract infection, nasopharyngitis, urinary tract infection, and bronchitis (other important adverse reactions include infusion reactions, serious infections, and cardiovascular events)
- GPA and MPA(≥ 15 %): infections, nausea, diarrhea, headache, muscle spasms, anemia, peripheral edema (other important adverse reactions include infusion reactions)

Rituximab administration can result in serious, including fatal infusion reactions. Deaths within 24 hours of Rituximab infusion have occurred. Approximately 80% of fatal infusion reactions occurred in association with the first infusion. Close monitoring of patient is required. Discontinue rituximab infusion for severe reactions and provide medical treatment for Grade 3 or 4 infusion reactions

IMMUNOGENICITY

As with all therapeutic proteins, there is a potential for immunogenicity. The observed incidence of antibody (including neutralizing antibody) positivity in an assay is highly dependent on several factors including assay sensitivity and specificity, assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to Rituximab with the incidence of antibodies to other products may be misleading. As per the reports using an ELISA assay, human anti-chimeric antibody (HACA) was detected in 4 of 356 (1.1%) patients with low-grade or follicular NHL receiving single-agent rituximab. Three of the four patients had an objective clinical response. A total of 273/2578 (11%) patients with RA tested positive for HACA at any time after receiving rituximab. HACA positivity was not associated with increased infusion reactions or other adverse reactions.

Upon further treatment, the proportions of patients with infusion reactions were similar between HACA positive and negative patients, and most reactions were mild to moderate. Four HACA positive patients had serious infusion reactions, and the temporal relationship between HACA positivity and infusion reaction was variable.

Conclusion

The development and introduction of recombinant monoclonal antibodies creates several opportunities and challenges. Physicians, biopharmaceutical companies, regulatory agencies and health authorities should collaborate closely to ensure equal efficacy and safety of recombinant monoclonal antibodies that will allow the accessibility of these powerful agents to a broader number of patients in less privileged areas of our planet.

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