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Mechanisms and Clinical Effects of Rituximab and its Multifaceted Comparison with Biosimilars

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Abstract:

Rituximab, targeting CD20, is one of the first-line treatments against Diffuse Large B-cell lymphoma (DLBCL), Follicular Lymphoma, Lymphoplasmacytic Lymphoma (LPL), and other B cell mutations. As the patent on Rituximab expires and a large number of biosimilars are on the market, Rituximab is an important milestone in the era of immunotherapy as the first monoclonal antibody to be approved for oncology treatment. The mechanisms of its anticancer effects include Complement-Dependent Cytotoxicity (CDC), Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC), Antibody-Dependent Phagocytosis (ADCP), direct signaling-induced cell death, etc. The biosimilar Truxima, which is now used worldwide, is also tending to surpass it by the expiration of the original Rituximab patent. This paper focuses on the working principle and clinical effects of Rituximab, the characteristics and pathway principles of the drug target CD20, as well as the physical and chemical properties of reference drugs and biosimilars. This review also summarizes the importance of monoclonal antibody technologies such as Rituximab and CD20 antibodies in the treatment of related cancers. Further studies are required for clinical applications of Truxima as a substitute for Rituximab.

Keywords: Rituximab, CD20, Truxima, signaling pathways, clinical trials.

1. Introduction

Epidemic therapy, as a rapidly developing quality method, is of great significance to the treatment of cancer. Unlike traditional drugs, epidemic therapy can activate the immune system to make B cells or T cells specific to the tumor. Monoclonal antibodies (mAb) have been one of the first approved immunotherapies for the treatment of tumors. One of the first monoclonal antibodies to be used in cancer treatment and is still widely used today is Rituximab.

Rituximab belongs to mouse and human chimeric mAb. In 1997, the world‘s first anti-CD20 monoclonal antibody, Roche‘s rituximab (trade name: Rituximab), was also the world‘s first marketed anti-tumor monoclonal antibody for the treatment of relapsed or refractory indolent lymphoma. Rituximab pioneered targeted therapy for lymphoma, and prior to rituximab, traditional chemotherapy drugs had been the first-line treatment for lymphoma. Currently, the combination of rituximab with chemotherapy agents CHOP and CVP is the standard treatment for diffuse large B-cell lymphoma and follicular lymphoma, respectively. The development of rituximab has undergone three generations of evolution. Currently, the third generation of rituximab (trade name: Gazyva, also known as GA101) is modified by glycosylation of the Fc segment, thereby improving the specificity of the antibody and the affinity for binding to the antigen. The reference drug for the biosimilar Truxima described in this article is the first-generation Rituximab [1].

One of the most significant problems in the development of tumor mAb therapy is target selection. If the receptor of the drug appears not only on the tumor cells but also on normal cells in large numbers, it would be toxic. CD20 is expressed on the surface of developing B.lymphocytes and a number of B.lymphocyte malignancies, but not mature plasma cells and B.lymphocyte progenitors. Therefore, in order to prevent side effects that occur due to the killing of ordinary B cells, it is possible to focus on CD20 [1]. CD20 is also related to B-cell receptor signaling and microenvironmental interactions [2].

This review summarizes the mechanisms of Rituximab, such as antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), antibody-dependent phagocytosis (ADCP) cell death and direct signaling-induced cell death, and clinical effects.

2. Mechanism of Rituximab and Truxima

Truxima is a CD20-specific antibody that kills tumor cells

by acting on ADCC, CDC, ADCP, and direct signaling-induced cell death (Figure 1). Moreover, it is one of the current therapeutic monoclonal antibody-targeting peptides [3].

2.1 Anti-CD20 Effects

CD20 is a glycosylated transmembrane phosphoprotein coded by the MS4A1 gene about 33-37 kDa. Because this protein is not expressed by mature plasma cells and B cell progenitors, the side effects associated with that target are acceptable [1]. Moreover, CD20 has an intracellular region and two extracellular loops. The out cellular parts are called small loop and large loop (ECL1 and ECL2), and ECL2 is longer than ECL1, with a disulfide bond.

CD20 is associated with B-cell receptor signaling and interactions in the microenvironment. Its use has been shown to be related to the control of calcium inflow in B cells and the B-cell receptor (BCR). Still, the mechanisms, whether it is through the activation of a signal or whether it is calcium channel protein itself, remain unclear. Normally, CD20 does not form heterooligomers but exists on the surface of the cell as homomeric and homotetrameric oligomers associated with other cell surfaces and cytoplasmic proteins that contribute to the transduction signal [2]. Although RTX has long been observed to form CD20 clusters on the surface of cells, newer studies have shown that RTX: CD20 combinations are similar in diameter to Fc hexamers [4].

2.2 Direct Signaling Induced Cell Death

Although directly killing cells is one of the mechanisms that are not fully understood, compared to well-known mechanisms such as acting through the immune system, there are still *in vitro* experiments to demonstrate the importance of directly induced cell death.

Direct cell killing can be categorized as caspase-dependent and independent cell death. A change in lipid rafts and CD20 localization to a type I CD20 antibody are some of the changes that occur when Rituximab binds to CD20 antibodies. The process is known to be dependent on family kinase src and results in caspase-mediated apoptosis [5].

2.3 CDC

Complement is from a group of non-thermostable proteins found in human or vertebrate serum and tissue fluid, including more than 30 soluble and membrane-bound proteins. The antibody first binds to complement C1q first, and then C2-C9 is activated to form a membrane-tapping complex to exert a lytic effect on the target cell. Many anti-tumor antibodies, such as anti-CD20, CD52, CEA, and other antibodies, can cause CDC action [6].

Studies have suggested that Complement-Dependent Cytotoxicity may have a negative effect on Rituximab (Truxima), which is mainly divided into two parts. The first reason is that the CDC will compete with ADCC for anti-CD20 antibodies, while the CDC is less effective than ADCC [7]. The second reason is that CDCC functions in humans around CD55 and CD59, and it is now shown that high expression of these proteins seems to have a positive correlation with resistance to Rituximab mAbs [8].

Overall, the anti-tumor mechanism of CDC and interactions between ADCC and ADCP with CDC have not been fully explained, and further *in vitro* and *in vivo* experiments are needed to better elucidate the mechanisms.

2.4 ADCC

ADCC refers to the Fab segment binding to the epitope of virus-infected cells or tumor cells, and the constant region of the antibody binds to the T cell, mediates killer cells‘ direct killing of target cells, and is considered an important mechanism for Ttuxima treatment of tumor cells.

The binding of the Truxima Fab fragment to CD20 will help the Fc fragment to FcγRIII, and the immune protrusion of the binding region will make contact. Here, there are two ways to kill host cells for NK cells. The first one is the release of perforin. In the presence of higher concentrations of calcium, the perforin space structure changes, resulting in the ability to bind and insert into the cell membrane of the target cell, followed by forming holes and implementing this action [1]. There will be 3-4 perforin oligomerization. The oligomers then attract surrounding perforin and gradually join in, thus expanding to contain 10-20 perforin. Subsequently, holes on the membrane, including multiple large holes with a small aperture of 5-20 nm or even 50-160 nm, cause cell membrane permeability catastrophe. The catastrophe caused by increased cell membrane permeability causes target cell permeability dissolution, and with a large probability, leads to target cell death. The second approach is the release of a granzyme B that induces programmed cell apoptosis by an enzyme-dependent mechanism, for example, by direct cleavage of caspase [9].

2.5 ADCP

The mechanism is the least studied of the four known mechanisms of Rituximab effects. Moreover, there are challenges similar to those of ADCC *in vitro* measurements. Although there is no *in vivo* evidence of Rituximab-mediated ADCP in humans, some evidence of ADCP in mouse models has been demonstrated on the basis of the use of specific FcIVR on the macrophage to achieve treatment [10].

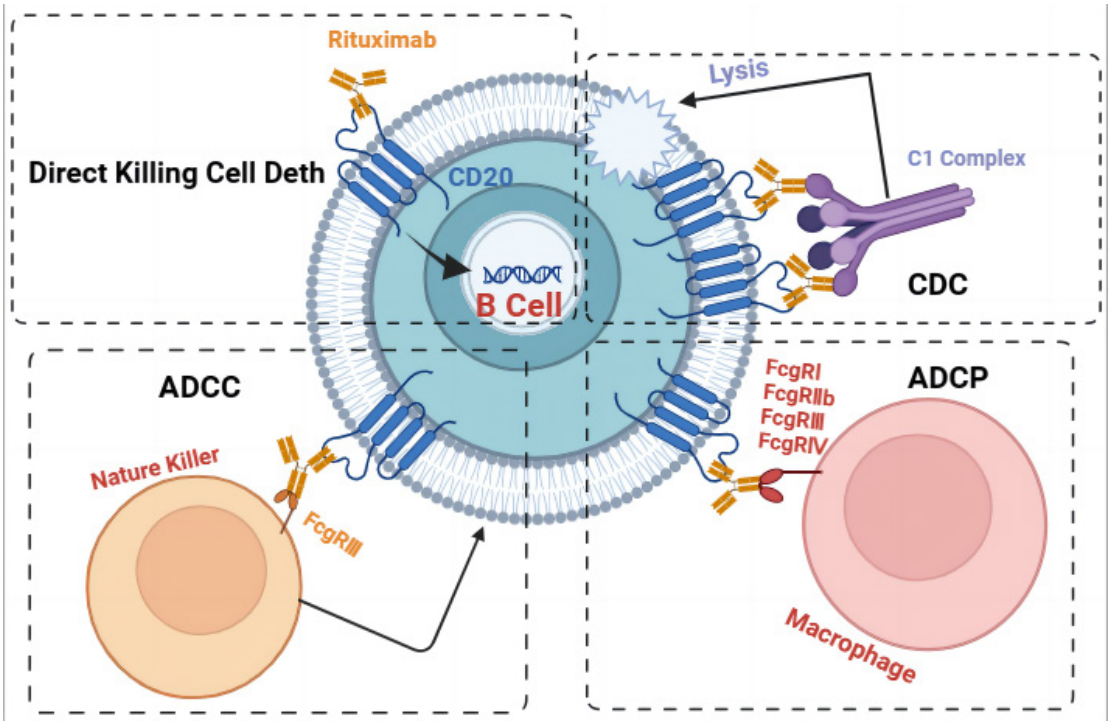

Fig. 1 Four mechanisms of Rituximab therapeutic effect: ADCC, ADCP, CDC, and Direct Signaling Induced Cell Death. Figure credit: original. Made from Biorender.com.

3. Pharmacokinetics of Rituximab

Global sales of Rituximab are on a downward trend, especially in 2018 after the expiration of the US patent. Through the price chart and share chart of Rituxiva products, truxima, as a biosimilar drug, has a declining price and increasing market share, which is conducive to the global sales of truxima. Through the SWOT analysis, truxima costs less but, like Rituximab, may have adverse effects on the immune system. Thus, more clinical trials are required in the future, but the expiration of Rituximab's patent will reduce its development cost and risk, which will be favorable for its development [3].

As a monoclonal antibody, Rituximab has more complex pharmacokinetics and pharmacodynamics, with non-linear dynamics, a long half-life of up to several weeks, and large individual differences in drug disposal. Moreover, related studies indicate that its efficacy may be influenced by various factors such as disease, tumor type, and genetic genes. Individual dosing studies of Rituximab are therefore necessary [11].

In recent years, population pharmacokinetics (PPK) has become one of the main tools for individualized clinical drug administration. It combines classical pharmacokinetics basic principles with statistical methods to study the characteristics of PK parameters of a group under a given dose regimen and sex on PK behavior.

In 2020, the Suzhou Institute of Nanotechnology and Nanobionics, Chinese Academy of Sciences, disclosed a method of mass spectrometry for pharmacokinetic studies of Rituximab. In order to reduce the influence of the sample processing process on the measurement results, the stable isotope-labeled peptide with a restriction site was designed and synthesized, and the labeled peptide was used as the internal standard peptide. This approach allows the ability to maximize the accuracy of protein sample determination. The plasma sample was analyzed by liquid chromatography-mass spectrometry by substituting the selected characteristic peptide peak area with the ratio of the internal standard peptide into the standard curve [12].

4. The Attribute Difference between Biosimilar and Reference Drug

The development of biosimilars is similar to the iteration of the reference drug, eventually resulting in a product that is highly similar to the original product. The research on biosimilars focused on comparing biosimilars to references to prove and confirm the biological similarity. One of the key factors is the extensive physicochemical and functional characterization.

4.1 Physicochemical characterization

Because the biosimilar drug mentioned in this article has high similarity to the reference drugs, such as primary structure, higher order structure, and size heterogeneity.

This part focuses on the glycosylation.
Minor glycans bNG1 and bG1S1 were identified in the originators when looking at the low-abundant glycan structures [13]. The proportion of mannose structures, such as Man 5, M7, etc., was also found to be slightly smaller in the bioequivalent. Overall, the biosimilar glycan profiles are similar to those of reference products [14].

4.2 Bioassays

Considering that Truxima has highly similar primary and advanced structures compared to the reference drug, the researchers used a bioassay to compare the bioequivalence of the two drugs. It is well established that in CDC and ADCC assays, a change in Fc glycosylation can affect the activity of mAbs. The latter assay has been shown to be highly sensitive when defucosylated glycans are present. In contrast to CDC, the variability of rituximab was significantly greater in ADCC assays.
The study compared the two drugs based on four parameters: target binding, ADCC, CDC, and apoptosis. The result showed significant differences regarding bioequivalence between the two drugs with the p-values (TOST) of all parameters less than 0.0001 [13].

5. Clinical Trials of Rituximab and Biosimilars

Rituximab is an immunoglobulin G1k antibody against four main target diseases, which can be indicated with different treatments. Non-Hodgkin's Lymphoma (NHL) is the direct target illness of this antibody. Chronic Lymphocytic Leukemia (CLL) can be treated in combination with chemotherapy. Rheumatoid Arthritis (RA) can be treated in combination with methotrexate [15].

5.1 Treatment for RA

In a Phase I clinical trial, patients were randomly injected with either CT-P10 or EU-RTX. The results showed high similarity between treatment groups in all endpoints. To have time for secondary treatment, the tristaff was extended to 72 weeks. The second courses of treatment were highly similar between the CT-P10 and originator Rituximab groups (Table 1).
A phase III study evaluated PK, PD, efficacy, and safety (Table 1). A total of 372 patients participated in this clinical trial, where 189 patients were randomized 1:1:1: CT-P10, EU-RTX, and US-RTX. In the second part, a total of 372 newly enrolled patients were divided into two groups: 162 in CT-P10 and 162 and 211 in EU-RTX plus US-RTX [16].

Table 1. Comparisons of biosimilars and references regarding therapeutic effects on Rheumatoid arthritis (RA)

Phase	Drugs	Results	References
Phase I	CT-P10 and EU-RTX	Highly comparable on efficacy endpoints	[16]
Phase III	CT-P10 and US-RTX/ EU-RTX	US-RTX/EU-RTX group and CT-P10 group were within a statistical equivalence margin in terms of estimated treatment differences, secondary efficacy outcomes, and side-effects.	[16]

5.2 Treatment for Follicular Lymphoma

The milestone phase II study included 37 FL patients, and 17 patients achieved a clinical response (which represents a 46% response rate). This fully demonstrated the high safety and high efficiency of Rituximab in response to FL and led to its approval. In 1998, the same dose of CT-P10 as in the test was used in a phase II / III multicenter trial as monotherapy, again achieving a 48% response rate [17]. Another milestone study to test the safety and feasibility of combining CHOP was published in 1999. The patients treated with RCHOP achieved an impressive 95% ORR and 55% CR, which helped reinforce RCHOP and other Rituximab combination treatments [18].

6. Conclusion

From the first successful manufacture of monoclonal antibodies in 1975 to FDA approval in 2013. In terms of market size, its global sales showed a downward trend, especially in 2018, after the expiration of the US patent. Truxima, as a biosimilar drug, keeps decreasing its price and increasing its market share, which is conducive to the global sales of truxima. Overall, the reference drug, rituximab, takes CD20 as the target and binds with the CD20 molecule expressed on the surface of B lymphocytes to kill tumor B cells. Rituximab binds to the large ring of the extracellular loop of CD20. Tumor B cells can be targeted and killed through several signaling pathways, including direct signaling-induced cell death, ADCC, CDC, and ADPH. Although the mechanisms of CD20 and Rituximab are still not fully understood, more research is required to get more useful information for the benefit of mankind in the near future. Truxima has a lower cost and similar efficiency and safety to Rituximab. However, Proxima

is likely to have adverse effects on the immune system. Thus, more clinical trials are needed in the future, but due to the expiration of Rituximab's patent, the reduction of development cost and risk would benefit its development.

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