Chapter

Monoclonal Antibodies for Cancer Treatment

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Abstract

Therapeutic monoclonal antibodies have emerged in the 1990 decade as an important option for cancer treatment. These molecules have a diverse set of clinically relevant antitumor mechanisms, directly targeting tumor cells. It has been established as "standard of care" for several human cancers. This chapter reviews the use of monoclonal antibodies in oncology and introduces available biosimilars. The requirements for biosimilar antibody development, mechanisms of action and current clinical applications for cancer treatment is also presented.

Keywords: biosimilar, equivalence trial, efficacy, monoclonal antibodies, cancer, extrapolation of indication

1. Introduction

Since the development of monoclonal antibodies by hybridoma technology in 1975 [1] over 80 molecules were developed and approved for therapeutic use in immunological, oncological, and infectious diseases [2]. Over time, these molecules have revolutionized the treatment of main autoimmune diseases and cancer that previously had a bleak prognosis. These molecules are usually administered by subcutaneous or intramuscular routes due to poor oral bioavailability (less than 1%) caused by large size, polarity, limited membrane permeability, and poor gastrointestinal stability [3].

In oncology, the approach in the use of monoclonal antibodies consists in targeting tumor antigens and killing cancer cells [4]. Growth factor receptors that are overexpressed in tumor cells are recognized as main target by monoclonal antibodies [4, 5]. Blocking ligand binding/signaling result in decrease growth rate of cancer cells, which in turn, induce apoptosis and sensitize tumors cells to chemotherapy [6, 7].

As of the first semester of 2021, the arsenal of monoclonal antibodies in oncology counts on more than 30 molecules [8]. Among the first molecules, we have: bevacizumab, cetuximab, rituximab, trastuzumab, indicated for treating solid tumors and hematological malignancies (**Table 1**). From all monoclonal antibodies, there are only three biosimilar products marketed (bevacizumab, rituximab, trastuzumab; **Table 2**).

Monoclonal antibody	Approval date		Mechanism of action	Indication in oncology
	EMA[a]	FDA[b]		
Bevacizumab	2005	2004	Inhibition of vascular endothelial growth factor binding to the cell surface receptors	Metastatic colorectal cancer; unresectable, locally advanced, recurrent, or metastatic non-squamous non-small cell lung cancer; recurrent glioblastoma in adults; metastatic renal cell carcinoma; persistent, recurrent, or metastatic cervical cancer; epithelial ovarian, fallopian tube, or primary peritoneal cancer; hepatocellular carcinoma
Cetuximab	2004	2004	Competitive inhibition of the binding of epidermal growth factor	Metastatic colorectal carcinoma
Rituximab	1998	1997	Binding to B-lymphocyte antigen CD20 on the surface of B cells and activating the antibody-dependent cellular cytotoxicity and apoptosis	Non-Hodgkin's lymphoma; lymphocytic leukemia
Trastuzumab	2000	1998	Binding to the human epidermal growth factor 2 (HER2) will result in inhibition of the proliferation and survival of the cell	HER2-overexpressing breast cancer; HER2-overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma

Legend: EMA, European of Medicines Agency; FDA, United States Food and Drug Administration; INN, international nonproprietary name.
[a] *Available at: www.ema.europe.eu.*
[b] *Available at: www.accessdata.fda.gov.*

Table 1.
First monoclonal antibodies used in oncology.

Monoclonal antibody	European of Medicines Agency[a]		Food and Drug Administration[b]	
	Trade name	Approval date	Trade name	Approval date
Bevacizumab	Mvasi	2018	Mvasi	2017
	Zirabev	2019	Zirabev	2019
	Equidacent	2020		
	Aybintio	2020		
	Onbevzi*	2020		
	Alymsys*	2021		
	Oyavas*	2021		
Rituximab	Truxima	2017	Truxima	2018
	Riximyo	2017	Ruxience	2019
	Blitzima	2017	Riabni	2020
	Rixathon	2017		
	Ritemvia	2017		
	Ruxience	2020		

Monoclonal antibody	European of Medicines Agency[a]		Food and Drug Administration[b]	
	Trade name	**Approval date**	**Trade name**	**Approval date**
Trastuzumab	Ontruzant	2017	Ogivri	2017
	Trazimera	2018	Herzuma	2018
	Kanjinti	2018	Kanjinti	2019
	Ogivri	2018	Ontruzant	2019
	Herzuma	2018	Trazimera	2019
	Zercepac	2020		

[a] *Available at: www.ema.europe.eu.*
[b] *Available at: www.fda.gov/drugs/biosimilars/biosimilar-product-information.*
[*] *The product received the recommendation of the granting of marketing authorization.*

Table 2.
Biosimilar monoclonal antibodies with marketing approval for cancer treatment (until February 2021).

2. Development of monoclonal antibodies

Monoclonal antibodies consist in homogenous preparations of antibodies – or fragments of antibodies – in which every antibody in the product is identical in its protein sequence. All antibodies should have the same antigen recognition site, affinity, biological interactions, and downstream biological effects [2].

There are four types of monoclonal antibodies [9]:

- Murine: entirely derived from a murine source (hybridoma technology).
- Chimeric: the variable regions are of murine origins whereas the constant regions are human.
- Humanized: mostly derived from a human source except for the part of the antibody which binds to its target.
- Human: entirely derived from a human source

In summary, the traditional murine hybridoma technique starts by immunization of mice with desired antigens to trigger an immune response. Harvested splenocytes are fused with myeloma cells to produce hybridoma cells that persistently secrete the antibodies of interest. After the screening, selected leads are used to generate chimeric or humanized antibodies [9].

The main concern with this approach is the risk that might result in an immune response to the mouse antibody sequence. The consequence of this include allergic response and/or reduced bioavailability of mouse monoclonal antibodies. This immune response limited their clinical use [10].

Changes in the source of the molecule were determined as a solution to avoid the immune response. Introducing engineer changes, for example, recombinant DNA technologies, originated the chimeric, humanized, and human antibodies. Humanized mice allow for development of monoclonal antibodies with amino acid substitutions that lack mouse heavy chains and make them more similar to the human sequence system [2, 9].

The first chimeric antibody was approved in 1994 by the United States Food and Drug Administration (FDA) for inhibition of platelet aggregation in cardiovascular

diseases. The drug was developed by combining sequences of the murine variable domain with human constant region domain. In 1997, the first monoclonal antibody, rituximab – an immunoglobulin type 1 anti-CD20 -, was approved for non-Hodgkin's lymphoma by the FDA [9]. And the first humanized monoclonal antibody approved by the FDA also in 1997 was daclizumab, an anti-IL-2 receptor used for the prevention of transplant graft rejection [11].

Human monoclonal antibodies can either be obtained by phage display or transgenic animals [9]. Based on these techniques, the first fully human therapeutic antibody based on phage display was adalimumab, an anti-tumor necrosis factor α human antibody. It was approved in 2002 by the FDA for rheumatoid arthritis. Panitumumab, a monoclonal antibodies anti-epidermal growth factor receptor was the first human antibody generated in a transgenic mouse, approved by the FDA in 2006 and indicated for metastatic colorectal carcinoma, a type of cancer [11].

3. Biosimilar monoclonal antibodies in oncology

As mentioned before, three biosimilar monoclonal antibodies are available in oncology: bevacizumab, rituximab, and trastuzumab. Cetuximab is in preliminary steps of developing a biosimilar.

Bevacizumab is a humanized inhibitor of vascular endothelial growth factor (VEGF) monoclonal antibody. It acts by selectively binding circulating VEGF, thereby inhibiting the binding of VEGF to its cell surface receptors, which results in a reduction of microvascular growth of tumor blood vessels, reducing the blood supply to tumor tissues. Other results observed are decrease interstitial pressure on tissues, increase vascular permeability, induction of apoptosis of tumor endothelial cells, and may increase delivery of chemotherapeutic agents [12].

Rituximab is a chimeric monoclonal antibody that has a high-affinity binding to B-lymphocyte antigen CD20 (CD20) on the surface of B cells. The death of B cells occurs by different ways, including antibody-dependent cellular cytotoxicity (ADCC) and apoptosis [13].

Trastuzumab is a recombinant humanized monoclonal antibody that binds to the domain of the extracellular segment of the human epidermal growth factor-2 receptor (HER2), and inhibits the proliferation and survival of HER2-dependent tumors [14]. When trastuzumab is biding to HER2 receptor might occur the degradation of the receptor, attraction of immune cells to tumor cells by ADCC and inhibition of some pathways involved in the suppression of cell growth and proliferation [15].

4. Assessment of biological activity of biosimilar monoclonal antibodies

The biosimilar needs to demonstrate the proposed product is highly similar to the reference biological product and this is determined through a pathway that include comparative characterization made by evaluation of physicochemical, functional, and clinical characteristics of a biological product [16, 17].

The first step in biosimilar analytic characterization is identifying the characteristics associated with the quality, safety, and efficacy of reference biological product. These characteristics are known as critical quality attributes (CQAs) and represent physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality [18].

Analytic testing of CQAs is performed to detect differences in factors such as the expression system, the manufacturing process, physicochemical properties, functional activities, receptor binding, immunochemical properties, impurities, and clinical outcome of the biosimilar candidate [19, 20].

It may be useful to compare the quality attributes of the proposed biosimilar product with those of the reference product using a meaningful fingerprint-like analysis. It means the results obtained are extremely sensitive in identifying analytical differences and allow a very high level of confidence in the analytical similarity of the proposed biosimilar product [21].

Once the CQAs for the biosimilar candidate are identified, the next step is to categorize the relative importance or criticality of each attribute. In the case of monoclonal antibodies, that are more complex biological products, determining criticality may be more challenging due to the increased number of attributes to evaluate and the potential impact of each difference on the desired product [22].

Significant differences for a very important CQA of the biosimilar candidate, such as the primary amino acid structure, are enough to interrupt the biosimilarity pathway. The manufacturer will need change their process to reach the high level of similarity between this structure in the biosimilar compared with the reference product. In the other hand, differences detected among CQAs of very low importance, such as minor modifications in amino acid side chains, may be acceptable if they can be justified or understood as clinically irrelevant [22, 23].

Primary amino acid structure is the core DNA sequence, and it must be exactly the same for the biosimilar product and the reference product [22]. There are a range of methods commonly used for evaluating the primary structure, including the peptide mapping, characterization of disulfide linkages, and glycosylation [24]. If the amino acid sequence is not identical, it can happen unwanted amino acid interactions that will impact in the safety, efficacy, and immunogenicity of the product [22].

Antibody molecules are molecules consisting of three equalized portions, constructed in the same way from paired heavy and light polypeptide chains that consists of a series of similar, sequences, each about more than a hundred amino acids long [25].

Changes in the protein can occur during any step of the manufacturing process, for example, enzymatic modifications, aggregation, variable glycosylation, etc. These modifications are named as post translational modifications. They can influence the physicochemical and biological properties of a protein and affect immunogenicity, immune response, and clinical efficacy [26]. In general, proteins can differ in at least three ways: (i) primary amino acid sequence; (ii) modification of amino acids, such as glycosylation or other side chains; and (iii) higher order structure [23]. Glycosylation and phosphorylation can impact on the efficacy and safety of a protein, for this reason, during the development process, they are extensively tested [22].

When the primary amino acid structure and the three-dimensional structure are reached in the biosimilar product, the correct protein arrangement and structural integrity are obtained and then, the ability of the biological product to bind to the target receptor will result in pharmacologic action. For this reason, target binding is considered a very highly CQA [27].

Impurities can be product – or process-related, arising from cell substrates or cell culture component [28]. They have the potential to affect all aspects of the product's profile [22]. For this reason, the chosen analytical procedures should be adequate to detect, identify, and accurately quantify biologically significant levels of impurities [28].

Because the quality attributes of a biosimilar are not identical to those of the reference product, in addition to the analytic package, animal toxicology,

pharmacokinetic and pharmacodynamic testing, and immunogenicity studies are required by the regulatory agencies for demonstrating biosimilarity [29]. Then, to ensure that these differences do not lead to any clinically meaningful differences, comparative clinical studies are performed [30]. It is usually necessary to demonstrate comparable clinical efficacy of the biosimilar and the reference product in adequately powered, randomized, parallel group comparative clinical trial(s), preferably double-blinded and appropriate endpoints chosen [19].

5. Requirements for biosimilar monoclonal antibody clinical trials

Since the first monoclonal antibody have come off patent protection, regulatory agencies like European of Medicines Agency (EMA), FDA, Health Canada, Australian government Therapeutic Goods Administration (TGA) as well as the World Health Organization (WHO), developed guidance to manufactures interested in submitting applications for biosimilar products approval. Principles for designing, conducting, and reporting the results from clinical trials are set by these guidelines.

Clinical pharmacology studies are a critical part of demonstrating biosimilarity by supporting a demonstration that there are no clinically meaningful differences between the proposed biosimilar product and the reference product [21].

The comparison of the pharmacokinetics properties of the biosimilar and the reference product forms the first step of a biosimilar monoclonal antibodies' development [29]. It is critical to use the appropriate bioanalytical methods to evaluate pharmacokinetics and pharmacodynamics properties [21]. They need to be accurate, precise, specific, sensitive, and reproducible.

The design of the study depends on some factors, including clinical context, safety, and the pharmacokinetics characteristics of the antibody [29]. Two study designs are of particular relevance: single dose crossover designs and parallel study designs. For pharmacokinetics similarity assessments, a single dose study, randomized, crossover study in healthy volunteers, is generally preferred [21, 29].

Pharmacokinetics and pharmacodynamics studies of trastuzumab (CT-P6 drug) [31] and bevacizumab (SB8 drug) [32] were developed with healthy participants. On the other hand, rituximab (PF-05280586) [33] were conducted with patients (rheumatoid arthritis or lymphoma). A study in healthy subjects is considered to be more sensitive in evaluating the product similarity because it is likely to produce less pharmacokinetics and/or pharmacodynamics variability compared with a study in patients with potential confounding factors [21].

Single dose study is recommended for a product with a short half-life, a rapid pharmacodynamics response, and a low anticipated incidence of immunogenicity [21]. To biological products with a long half-life, e.g., the mean serum half-life of rituximab is 59.8 hours after the first infusion [34], to evaluate clinical pharmacokinetics and pharmacodynamics similarity, a parallel group design is more appropriate for this kind of product [21, 29].

To demonstrate comparable clinical efficacy of the biosimilar and the reference product, an adequately powered, randomized, parallel group comparative clinical trial, preferably double-blind, by using efficacy endpoints is usually necessary [19].

Confirmatory trials (superiority trials) for new drugs should demonstrate that the investigational product provides clinical benefit. In this way, FDA and EMA have published guidance to applicants, providing background information and general regulatory principles for cancer clinical trials [7, 35]. Acceptable primary clinical endpoints in this kind of trial include cure rate, overall survival (OS), progression free survival (PFS), disease free survival (DFS) [7, 35].

While clinical trials of originator products aim to demonstrate patient benefit, in the biosimilar comparable studies the intention is to compare the biosimilar product with the reference product to exclude clinically relevant product-specific differences [36]. In this case, the most appropriate study design is the equivalence study, and in some specific cases, non-inferiority trial may be accepted after to discuss with regulatory authorities [19, 23, 29]. For this, the manufacturer needs justify on the basis of a strong scientific rationale.

OS is considered the most reliable cancer endpoint because is precise, easy to measure and the bias is not a factor to worried. It is defined as the time from randomization until death from any cause. It is measured in the intent-to-treat population [29, 35]. As it is necessary to perform the study with long follow-up periods in large trials, this endpoint is not usually expected to be present in the biosimilar studies and it is not required by the regulatory agencies.

In the comparable studies, it is not necessary to use the same primary efficacy endpoints as those that were used in the marketing authorization application of the reference product [19, 37]. However, EMA advises to include some common endpoints to facilitate comparisons to the clinical trials conducted with the reference product [19].

At moment, a large number of studies with bevacizumab, rituximab and trastuzumab biosimilar are using the ORR as the primary endpoint, and EFS, PFS as the secondary endpoint (**Table 3**). OS is less frequently used.

ORR is defined by the regulatory agencies as the proportion of patients with tumor size reduction of a predefined amount and for a minimum time period. The FDA has defined ORR as the sum of partial responses plus complete responses (CRs) [35]. ORR is a direct measure of a drug antitumor activity and should be assessed using a standardized criterion to determine the response [35]. The most common is the Response Evaluation Criteria in Solid Tumors (RECIST) guideline [55].

Beyond the pharmacokinetics and pharmacodynamics analyses, and clinical results, immunogenicity data should be collected and evaluated too. The goal is to investigate presence of an immune response to the therapeutic protein and its clinical impact [56].

The risk of immunogenicity varies between products and product categories, as well, between individuals and patient groups [56]. The consequences of an immune reaction to a therapeutic protein range from transient presence of anti-drug antibody (ADA) without any clinical significance to severe life-threatening conditions [56]. Immune responses to therapeutic protein products have the potential to affect product pharmacokinetic, pharmacodynamics, safety, and efficacy [56, 57].

When an ADA binds to or near the active site of a therapeutic protein or induces conformational changes, binding to relevant receptors will not happen and it will affect efficacy of the product. Besides these conformational-based effects, in addition immune-based adverse effects can happen. This includes injection-site and infusion reactions [56].

Among the product-related factors we have the protein origin (e.g. human or animal) and nature of the active substance (endogenous protein, post-translational modifications), significant modifications in the molecule structure, process-related impurities, formulation (excipients) and the interactions between the drug and/or formulation with the primary product packaging [56].

Immunogenicity testing of the biosimilar and the reference product should be conducted within the biosimilar comparability exercise by using the same assay format and sampling schedule which must meet all current standards [56, 58]. Assays used to detect antibodies against monoclonal antibody are often more problematic, difficult and can be technically challenging than for other proteins less complex [59].

Monoclonal antibody	Trade name	Study name or ID	Study design	Population and sample size (N)	Primary endpoint
Bevacizumab	Mvasi [38]	20120265	Non-inferiority study, randomized, double-blind, parallel, randomized	unresectable, locally advanced, or metastatic non-small cell lung cancer (642)	ORR
	Zirabev [39]	B7391003	Equivalence study, double-blind, parallel, randomized	unresectable, locally advanced, recurrent or metastatic non-squamous non-small cell lung cancer (719)	ORR
	Equidacent [40]	FKB238-002	Equivalence study, double-blind, parallel, randomized	Newly diagnosed advanced (stage IV) / recurrent NS-NSCLC (731)	ORR
	Aybintio [41]	SB8-G31-NSCLC	Equivalence study, double-blind, parallel, randomized	Metastatic or recurrent nonsquamous Non-small Cell Lung Cancer (763)	ORR
	Onbevzi	No information for public access			
	Alymsys				
	Oyavas				
Rituximab	Riabni [42]	20130109	equivalence study, randomized, double-blind, parallel	low tumor-burden follicular lymphoma (256)	ORR
	Ruxience [43]	REFLECTIONS B328-06	equivalence study, randomized, double-blind, parallel	low tumor burden follicular lymphoma (394)	ORR
	Truxima [44]	CT-P10 3.3	non-inferiority, randomized, double-Blind, parallel	Advanced Follicular Lymphoma (121)	ORR
	Riximyo [45]	GP13-301	Equivalence study, randomized, double-blind, parallel	previously untreated, advanced stage follicular lymphoma (629)	ORR
	Blitzima [46]	CT-P10 3.3	non-inferiority, randomized, double-blind, parallel	Advanced Follicular Lymphoma (121)	ORR
	Rixathon [47]	GP13-301	Equivalence study, randomized, double-blind, parallel	previously untreated, advanced stage follicular lymphoma (629)	ORR
	Ritemvia [48]	CT-P10 3.3	non-inferiority, randomized, double-blind, parallel	Advanced Follicular Lymphoma (121)	ORR

Monoclonal antibody	Trade name	Study name or ID	Study design	Population and sample size (N)	Primary endpoint
Trastuzumab	Ogivri [49]	MYL-Her-3001	equivalence study, randomized, double-blinded, parallel	metastatic breast cancer (458)	ORR
	Trazimera [50]	B3271002	equivalence study, randomized, double-blind, parallel	metastatic breast cancer (707)	ORR
		B3271004	non-inferiority study, randomized, double-blind, parallel	operable breast cancer (226)	pCR
	Kanjinti [51]	20120283	equivalence study, randomized, double-blind, parallel	operable breast cancer (725)	pCR
	Ontruzant [52]	SB3-G31-BC	equivalence study, randomized, double-blinded, parallel	operable breast cancer (875)	pCR
	Herzuma [53]	CT-P6 3.2	equivalence study, randomized, double-blind, parallel	operable breast cancer (549)	pCR
	Zercepac [54]	HLX02-BC01	equivalence study, randomized, double-blind, parallel	metastatic breast cancer (649)	ORR

Legend: ORR, overall response rate; pCR, pathological complete response.

Table 3.
Study design and primary endpoint for biosimilar monoclonal antibodies for cancer treatment.

Finally, when all tests are done and the authorization holder will submit the documents to receive the marketing authorization, it can be extrapolating all indications from the reference product to the biosimilar. When biosimilar comparability has been demonstrated in one indication, extrapolation of clinical data to other indications of the reference product could be acceptable but needs to be scientifically justified. It is expected that the safety and efficacy can be extrapolated when biosimilar comparability has been demonstrated in all aspects described before [19, 23, 29].

This condition is not applied in all situations. For example, if a reference monoclonal antibody is licensed both as an immunomodulator and as an anticancer antibody, the scientific justification as regards extrapolation between the two indication is more challenging and may have to involve more specific studies [29].

6. Conclusions

Since monoclonal antibodies play an essential role in cancer treatment and are responsible for high healthcare costs, the development of biosimilars is particularly important in oncology. Several biosimilars of the monoclonal antibodies trastuzumab, rituximab, and bevacizumab have been approved and began to be marketed in Europe, EUA and other countries around the world. More diversification of monoclonal antibodies biosimilars is expected in the next years, as the patent of other molecules will expire.

The biosimilar development pathway consists of a comprehensive comparability exercise between the biosimilar candidate and the reference product, primarily focusing on data from analytical studies. Clinical studies for biosimilar candidates follow a different design to those for a new biological. Adequate information on the biosimilar approval pathway, the robustness of overall evidence used to demonstrate biosimilarity, and how the clinical development of a biosimilar is done is important for all: professional, patient, governments, and other stakeholders.

Conflict of interest

Annemeri Livinalli: is involved in consulting, advisory work and speaking engagements for Amgen, Sandoz, Teva, Servier, Dr. Reddy's, Accord, United Medical, Achè.

Taís Freire Galvão: The author declares no conflict of interest.

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