Scientific rationale underpinning the development of biosimilar rituximab in hematological cancers and inflammatory diseases

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Sandoz rituximab (SDZ-RTX; Rixathon®; GP2013), a rituximab biosimilar, was approved in June 2017 in Europe in all indications of reference rituximab. The stepwise SDZ-RTX development program generated extensive physicochemical, structural, functional, and biological data demonstrating a match with reference rituximab on all clinically relevant attributes. A focused clinical development program followed, in two indications selected for sensitivity to detect potential differences versus reference rituximab: rheumatoid arthritis (pivotal pharmacokinetics and efficacy evaluation) and follicular lymphoma (pivotal efficacy/safety evaluation). These trials demonstrated highly similar pharmacokinetics, pharmacodynamics, efficacy, safety, and immunogenicity profiles. The totality of evidence for biosimilarity for SDZ-RTX, combined with knowledge that B-cell depletion is common to each approved indication, allowed SDZ-RTX approval for use in all indications of reference rituximab.

First draft submitted: 22 July 2019; Accepted for publication: 15 August 2019; Published online: 13 November 2019

Keywords: analytical • biosimilar medicines • clinical • non-Hodgkin lymphoma • preclinical • rheumatoid arthritis • rituximab • SDZ-RTX • totality of evidence

A biosimilar is a biologic medicine that matches an existing approved biologic, known as the reference medicine. A biosimilar matches the reference medicine in terms of safety, efficacy and quality, and physicians and patients can expect the same clinical behavior from the biosimilar as the reference medicine. Biosimilar medicines are developed and approved according to specific and stringent regulatory pathways [1]. Both the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) recommend a stepwise approach to development [2,3], with each step building on previous data to provide clear evidence of the similarity of the two medicines.

The first step is to establish biosimilarity at the molecular level, including physicochemical, structural, functional, and biological analyses. The robust evidence of similarity generated across this extensive battery of tests provides the scientific justification for a selective and targeted approach to animal and clinical testing [3], in contrast to the comprehensive testing required for a novel molecule. In 2009, when the clinical development program for Sandoz rituximab (SDZ-RTX; Rixathon®) was designed, animal testing was a regulatory requirement for biosimilar medicines. The animal testing studies for SDZ-RTX were chosen and agreed upon in collaboration with the EMA. Over the past decade, the requirement for animal testing has been relaxed. As such, testing of toxicity, pharmacokinetics and pharmacodynamics (PK/PD) in animals may be performed, but is no longer a prerequisite in the development process for biosimilars. Such testing is generally only necessary in cases where residual uncertainty

remains after initial analytical testing. Preliminary, Phase I PK/PD and immunogenicity evaluations may also be conducted in humans prior to the final step: a clinical trial to confirm efficacy and safety in an indication sensitive to detect any potential differences between the two medicines. The evidence of similarity at the analytical, preclinical, and clinical level gathered throughout the development program is also known as the totality of the evidence for biosimilarity.

In this review, we provide an overview of the scientific and regulatory principles underpinning biosimilar approval, using SDZ-RTX as an example, which will act as a useful addition to previous similar articles focusing on other biosimilars for oncology [4,5].

Characteristics of rituximab

Rituximab is a chimeric IgG1 monoclonal antibody binding with high specificity and affinity to CD20, a cell surface antigen present on normal and malignant B cells during cell maturation and differentiation [6–11]. Rituximab is approved for treatment of non-Hodgkin's lymphoma (NHL; follicular lymphoma [FL] and diffuse large B-cell lymphoma [DLBCL]), chronic lymphocytic leukemia (CLL), rheumatoid arthritis (RA), granulomatosis with polyangiitis (GPA), and microscopic polyangiitis (MPA). In all of these indications, rituximab has a central mode of action involving depletion of B cells [12]. Several different effector functionalities contribute to the central mode of action, with varying contributions in different indications. The primary functionalities are thought to be antibody-dependent cellular cytotoxicity (ADCC), triggered upon binding of the Fc portion of rituximab to Fc γ -receptor III-bearing natural killer (NK) effector cells, and CDC, a lytic cascade triggered through binding of C1qA to the Fc-domain of rituximab [6,7]. In addition, phagocytosis of malignant B cells by macrophages (antibody-dependent cellular phagocytosis [ADCP]) and programmed cell death (apoptosis), mediated via CD20 cross-linking and clustering, are also believed to contribute to rituximab-mediated B-cell depletion [7]. Crucially, the shared involvement of B cells in the pathogenesis of all approved rituximab indications provides a solid foundation for clinical experience with reference rituximab to be applied to biosimilar rituximab in indications that have not been directly tested, in a common regulatory process known as extrapolation.

Development of SDZ-RTX: a rituximab biosimilar

Biosimilar approval via extrapolation in non-tested indications is only possible with strong scientific justification – that is, data supporting biosimilarity. These data are provided by the totality of evidence, arising from structural, physicochemical, functional, and biological characterization of the biosimilar, followed by targeted animal testing (if residual uncertainty remains) and clinical trials. The evidence is generated via a multistep pathway consisting of five main stages: 1. Define the target profile, based on the reference medicine (identical amino acid sequence); 2. Define structural and functional quality attributes and evaluate their variability in the reference medicine; 3. Develop the biosimilar to match predefined target profile (systematic engineering of proposed biosimilar to match the reference medicine across development of cell line, bioprocesses, and drug product); 4. Physicochemical and biological characterization to demonstrate biosimilarity; 5. Regulatory interactions to determine clinical study testing requirements to confirm biosimilarity in suitably sensitive indications to obtain approval [13].

The development program for SDZ-RTX (Table 1) followed the multistep evidence generation pathway outlined above, resulting in a rituximab biosimilar end product that is similar to the reference medicine in all relevant attributes [14].

Target-directed development

Prior to initiating development of a biosimilar, it is essential to define the target parameters, known as critical quality attributes, via analysis of the reference medicine. This is achieved using an extensive and comprehensive battery of extremely sensitive state-of-the-art methodologies to analyze more than 100 different quality attributes [13]. These include physicochemical attributes (protein structure, post-translational modifications, charge variants, level and type of impurities) and biological attributes (antigen and Fc-receptor binding affinities, and performance in functional bioassays reflecting all effector functionalities contributing to the central mechanism of action). Multiple batches of the reference medicine must be studied to determine the variability of the reference molecule over time and from batch to batch [15,16], which establishes the target range, or goal posts, for each of the physicochemical and functional attributes for development of the biosimilar [17]. It is important to evaluate and rank the various attributes in terms of their criticality, which is determined according to the potential clinical impact in terms of

Table 1. SDZ-RTX development program.

Stage of development	Class	Outline
Analytical development	Physicochemical characterization	Primary structure, higher order structure, size; heterogeneity (C and N termini); post-translational modifications (variants, glycosylation, glycation, oxidation, deamidation); purity, aggregates
	Biological characterization	Binding (to CD20 target and Fcγ receptors); mode of action: ADCC, CDC, induction of apoptosis and ADCP
Preclinical development	<i>In vivo</i>	PK/PD (single and repeat dose in monkeys); toxicity (repeat dose in monkeys); efficacy (xenograft tumor models in SCID mice)
	<i>In vitro</i>	Tissue cross reactivity; ADCC (using primary PBMCs and whole blood)
Clinical development	Rheumatoid arthritis	Primary objective: PK (bioequivalence of AUC) Secondary objectives: additional PK parameters, PD, efficacy and safety of SDZ-RTX and reference rituximab in subjects with RA
	Advanced follicular lymphoma	Primary objective: efficacy – equivalence of ORR (primary endpoint) between SDZ-RTX and reference rituximab Secondary objectives: additional efficacy parameters, PK, PD and safety

ADCC: Antibody-dependent cellular cytotoxicity; ADCP: Antibody-dependent cellular phagocytosis; AE: Adverse event; AUC: Area under the plasma concentration–time curve; CDC: Complement-dependent cytotoxicity, FL: Follicular lymphoma; ORR: Objective response rate; PBMC: Peripheral blood mononuclear cell; PD: Pharmacodynamic; PK: Pharmacokinetic; RA: Rheumatoid arthritis; SCID: Severe combined immunodeficiency.

Table 2. Structural and physicochemical characterization of SDZ-RTX.

Comparison	Result
Intact mass analysis	Heavy and light chains showed exact expected molecular mass [20]
Peptide mapping for amino acid sequence analysis	Identical amino acid sequences Identical disulphide bridging patterns [20]
Secondary and tertiary structure	Several statistically independent methods revealed that the secondary and tertiary structures are indistinguishable [20] Nuclear magnetic resonance spectra were superimposable [20] Thermodynamic stability was comparable [20]
Charge variants	Similar levels of 0K variant and basic variants; acidic variants slightly below the reference medicine range [20]
Specific amino acid modifications	Similar levels of deamidated and oxidated peptides [20] Comparable presence of glycosylated species [20]
Post-translational modifications	Highly comparable glycosylation pattern, including major abundant glycans such as the bG0, bG1 (1,6.bG1 and 1,3.bG1) and bG2 isoforms [21–23] Range for afucosylated bG0 was within range of reference medicine (0.3–1.8%) [20] Small differences in low abundance glycan structures with limited clinical relevance [20]
Size heterogeneity	Comparable with reference medicine [20]
Purity	Very low aggregate levels [20] Within regulatory limits for subvisible particles [20] Practically free of visible particles [20]

PK/PD, efficacy, safety, and immunogenicity. Biosimilar development focuses on achieving the closest possible match for attributes with very high criticality scores when selecting the final expression cell clone and manufacturing process. The first step is to develop a gene construct suitable for expression of the same amino acid sequence as the reference medicine [18]. A process of clone selection is then performed, applying physicochemical and functional characterization techniques to assess the ability of the clone to produce an antibody molecule closely matching the reference medicine, before further selection and refinement steps, resulting in a final clone for production [19].

Physicochemical and biological characterization

Following definition of target parameters for SDZ-RTX, a manufacturing process for the candidate biosimilar was established via re-iterative development, considering all process steps, to ensure production of a biosimilar that matched the target parameters and quality defined by the reference medicine – that is, a ‘target-directed’ strategy as outlined above.

A comprehensive array of analytical techniques was employed to demonstrate that the final expression clone and manufacturing process of SDZ-RTX resulted in structural and physicochemical characteristics that match those of reference rituximab (Table 2) [20].

While essential, an indistinguishable protein structure alone is not sufficient to guarantee similarity of biological function, as many protein functions are influenced significantly by post-translational modifications, principally

glycosylation [24]. The level and pattern of these modifications depends greatly on the expression host cell line, and can also be influenced by the production process, and so in turn these modifications are highly variable amongst different biologics [25]. Analysis of post-translational modifications for SDZ-RTX demonstrated a highly comparable glycosylation pattern to the reference medicine (Table 2). In particular, afucosylated glycan structures were a key focus, as these have a profound influence on ADCC activity, one of the key effector functionalities contributing to the mechanism of action of rituximab [12]. SDZ-RTX was designed to contain approximately 1.4% of afucosylated bG0, which is within the range of 0.3–1.8% that has been shown for the reference medicine [20]. For several other glycan structures, variations are known to be less clinically relevant for rituximab, and so less stringent reference range criteria can be applied if adequately justified, as was seen with low-abundance glycan species for SDZ-RTX [20].

Functional characterization: in vitro bioassays

Following demonstration of matching structural and physicochemical properties between SDZ-RTX and reference rituximab, the next step was to demonstrate that the two medicines match with regard to functional and biological activity, focusing principally on B-cell depletion. Each of the effector functionalities contributing to the mechanism of action of rituximab are dependent upon binding to the CD20 target antigen on the surface of B cells [12]. Cell-based bioassays demonstrated that SDZ-RTX has similar CD20 binding compared with reference rituximab in all ascribed effector functionalities contributing to the mechanism of action [20]. Effects mediated by CD20 binding are complemented by affinity for Fc gamma receptors (FcγR) expressed on effector cells (FcγRIa, FcγRIIa, FcγRIIb, FcγRIIIa F158, FcγRIIIa V158, and FcγRIIIb), which are implicated in exerting anti-B cell activity resulting in B-cell depletion [12]. In addition, binding to the neonatal Fc receptor (FcRn) has an impact on the PK properties of rituximab, by protecting the antibody from intracellular catabolism [26]. Binding of the Fc-domain of SDZ-RTX and reference rituximab to Fc receptors was extensively assessed, demonstrating comparable affinity for these receptors between SDZ-RTX and reference rituximab [20].

Further information on function was obtained via cell-based bioassays to directly assess the known effector functionalities of rituximab – that is, ADCC, CDC, induction of apoptosis and ADCP. Results from these potency bioassays showed a match between SDZ-RTX and reference rituximab as the range of activity was comparable for all [20]. Matching ADCC potency of SDZ-RTX and reference rituximab was confirmed in other *in vitro* assays under more physiological conditions using two additional lymphoma cell lines and freshly purified NK cells [27], or using a lymphoma cell line with fresh PBMCs as effector cells [14]. Further confirmatory information was provided by an *in vitro* whole blood assay reflecting the additive effects of all effector functionalities. The concentration-dependent decrease of B cells in these assays was similar for SDZ-RTX and reference rituximab [14]. Overall, functional evaluations of SDZ-RTX demonstrated that it is highly similar to reference rituximab.

Preclinical and clinical studies

Once biosimilarity is established at a physicochemical, functional and biological level, additional animal studies may be necessary to assess exposure, efficacy, and toxicity and to confirm biosimilarity prior to the initiation of human studies [2,3]. As the SDZ-RTX preclinical development program began in 2006, it is important to consider that the program was designed according to the relevant guidelines available at the time, as well as direct feedback from health authorities. Since then, guidelines have been updated to further emphasize the ‘3Rs principle’ [28] and *in vivo* animal studies are only required in cases where they can sensitively address any remaining residual uncertainty. Comparative human PK/PD studies and clinical immunogenicity studies are also required, and usually at least one confirmatory Phase III clinical efficacy and safety study in a sensitive indication is necessary to confirm therapeutic equivalence and eliminate any residual uncertainties prior to approval. However, the concept of extrapolation means that there is no requirement to conduct additional confirmatory clinical efficacy and safety studies in all approved indications of the reference medicine, as that has already been established for the reference medicine. Overall, the clinical trial program for a biosimilar should be guided by discussions with regulatory agencies.

For SDZ-RTX, preclinical PK/PD analysis was performed in cynomolgus monkeys, in which single-dose administration of SDZ-RTX or reference rituximab showed comparable results, with nearly identical AUCs and 90% CIs within the standard bioequivalence acceptance range of 0.8–1.25 [27]. Noncompartmental PK analysis confirmed the bioequivalence of SDZ-RTX and reference rituximab. To test comparative efficacy under different sensitivity settings, bioequivalence between SDZ-RTX and reference rituximab was also assessed in two cynomolgus monkey B-cell subpopulations with differing levels of CD20 expression and differing susceptibility to rituximab-

induced depletion. Although minor differences were detected, overall good similarity was confirmed in both subpopulations across the various single dose levels tested [27]. Repeated doses of SDZ-RTX or reference rituximab given to cynomolgus monkeys also produced comparable PD profiles with pronounced depletion of the two peripheral B-cell subpopulations analyzed with both 20 mg/kg and 100 mg/kg doses. A slow return to baseline level was observed only in the 20 mg/kg groups. This repeat-dose toxicity study in cynomolgus monkeys also confirmed comparable exposure with SDZ-RTX and reference rituximab throughout the observation period, as well as a similar safety profile with no unexpected adverse effects [27]. Anti-tumor activity of SDZ-RTX and reference rituximab was assessed in two xenograft severe combined immune deficiency mouse models of NHL. To further increase the sensitivity of the models to identify any potential differences between SDZ-RTX and reference rituximab, suboptimal therapeutic doses were tested. Inhibition of tumor growth *in vivo* was comparable for SDZ-RTX and reference rituximab at all four different sensitive dose levels investigated [27]. Overall, preclinical *in vitro/in vivo* PK/PD and toxicity studies showed that SDZ-RTX and reference rituximab are highly similar.

In the stepwise development process for biosimilars, following the establishment of biosimilarity at the structural, physicochemical, functional, and preclinical levels, clinical studies are conducted to confirm that the candidate biosimilar and reference medicine are also comparable with regard to efficacy, safety, and immunogenicity. Regulatory guidelines stipulate that biosimilars should be evaluated in a sensitive indication – a disease where available clinical endpoints are sensitive enough to detect any clinically relevant differences in efficacy and safety, should there be any. For evaluation of a biosimilar rituximab, an ideal sensitive endpoint should show a large difference in effect size when rituximab is added to standard therapy. Clinical safety is a particularly important endpoint for assessing the biosimilarity of complex biological medicines, where immunogenicity and infusion-related reactions must be considered in addition to general safety/tolerability as measured by adverse events (AEs).

Rituximab has been widely studied both in previously untreated, advanced FL, and in RA, and considerable clinical data are available for comparative purposes in these two different indications that span malignant and nonmalignant disease. For the clinical development of SDZ-RTX, clinical trials in these two sensitive indications were performed [29,30]. RA was chosen as a sensitive indication for evaluation of PK/PD because of lower between-patient variability and lower, more homogenous baseline B-cell counts compared with oncology indications. Furthermore, the dosing regimen (24 weeks between courses) allowed the complete drug concentration–time profile to be captured. ASSIST-RA was the pivotal trial designed to demonstrate PK/PD equivalence, as well as similar efficacy and safety (including immunogenicity), between SDZ-RTX and reference rituximab in patients with RA [30]. The trial met its primary objective of demonstrating PK bioequivalence between SDZ-RTX and reference rituximab (Table 2) [30], and also demonstrated PD equivalence, as measured by the depletion of peripheral B cells. Similarity between SDZ-RTX and reference rituximab was additionally shown using multiple efficacy outcomes, including the powered efficacy objective, DAS28-CRP (disease activity score in 28 joints with CRP) (Figure 1). The rates of any AE, AEs related to study drug, AEs leading to drug discontinuation, and serious AEs (SAEs) were similar between treatment arms. Binding antidrug antibodies (ADAs) were detected in 16.5% of patients treated with SDZ-RTX versus 15.1% of patients who received reference rituximab. Most ADAs (10.2% and 9.6%, respectively) were transient, meaning that the patient had an ADA-negative sample after having an ADA-positive sample(s). ADAs with neutralizing capacity were rare (3.9% for the SDZ-RTX group and 0.6% for the reference rituximab group) and were mostly transient [30].

In consultation with regulatory authorities, FL was selected as a sensitive indication for the pivotal efficacy and safety study, as the addition of rituximab to chemotherapy in this population has been shown to provide substantial additive treatment effects compared with chemotherapy alone, thereby making it a sensitive indication to detect potential differences. Overall response rate (ORR) was considered the most sensitive endpoint to detect differences between treatments [29], given the large effect size on this endpoint when rituximab was added to the chemotherapy backbone of cyclophosphamide, vincristine, and prednisone (CVP) in the M30921 study in patients with advanced FL [31]. ASSIST-FL was a Phase III, confirmatory trial in patients with untreated advanced FL [29]. The aim was to confirm equivalent efficacy and comparable safety, PK/PD, and immunogenicity, between SDZ-RTX and reference rituximab when used in combination with CVP. As both 95% CI (-5.94 to 5.14) and 90% CI (-5.10 to 4.30) for ORR lay entirely within the predefined equivalence interval of $\pm 12\%$ (Figure 2), the trial met its primary endpoint of showing equivalence between SDZ-RTX and reference rituximab. Additionally, PK, PD, safety, tolerability, and immunogenicity outcomes supported similarity between the two medicines.

Figure 1. Change from baseline in disease activity score DAS28 CRP from baseline to Week 24 in patients with rheumatoid arthritis treated with SDZ-RTX or reference rituximab for 24 weeks.

DAS28, disease activity score in 28 joints.

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Figure 2. Primary efficacy analysis of overall response rate (CR or PR) based on central blinded review (PPS) in patients with untreated advanced follicular lymphoma treated with SDZ-RTX or reference rituximab in combination with cyclophosphamide, vincristine, and prednisone (CVP).

CR: Complete response; PPS: Per protocol set; PR: Partial response.

Created using data from [29].

Equivalence in clinical pharmacological properties demonstrated in RA, supported by ORR equivalence in FL, as well as similarity in further efficacy, safety, and immunogenicity end points, provides confirmatory clinical evidence of the biosimilarity of SDZ-RTX with reference rituximab [29,30].

Implications for extrapolation with SDZ-RTX and reference rituximab

Two key criteria must be satisfied to allow approval of a biosimilar in the same indications as the reference medicine by extrapolation [2,3]. The first of these is the totality of evidence for biosimilarity derived from the comprehensive comparability exercise between the two medicines, including structural, physicochemical, functional, biological, pharmacological, and immunogenicity data [2,3]. As described in this manuscript, the development of SDZ-RTX involved an extensive program of studies to support this evidence, all of which demonstrated a match between SDZ-RTX and reference rituximab across a comprehensive set of parameters, including those that are critical to the pharmacological and clinical profile of rituximab.

The second criterion is demonstration of clinical similarity in an indication that is both representative of the intended clinical use of the medicine and sufficiently sensitive to be able to detect clinically relevant differences in efficacy and safety, should there be any [2,3,32]. As noted previously, untreated advanced FL is a sensitive indication for comparison of efficacy for SDZ-RTX and reference rituximab, and RA is a sensitive clinical indication for comparison of PK/PD and immunogenicity. Data from these two indications provided strong evidence of similarity on a clinical level. These data, along with the totality of evidence for biosimilarity, allowed extrapolation of data from the reference medicine for the other approved indications (DLBCL, CLL, GPA, and MPA), removing the need for clinical trials in these additional indications.

A third criterion must be considered when the mechanism of action is complex and involves multiple receptors or binding sites, the relative contribution of which may differ between indications or may be uncertain [33]. Additional *in vivo* functional or PD data may then be required to provide evidence that the biosimilar and reference medicine will act in a similar way across extrapolated indications [34]. Rituximab is known to act via depletion of B cells mediated through CD20 binding in all approved indications. In addition, the effector functionalities contributing to this overarching mode of action have been extensively characterized and are known to play a role across all indications [12]. Critically, a wide variety of *in vitro* and *in vivo* functional and PD studies have been conducted to confirm that SDZ-RTX and reference rituximab are matching with regard both to the overarching mechanism and to the key effector functionalities driving this mechanism.

Therefore, the data for SDZ-RTX satisfy all relevant criteria for extrapolation. As such, in June 2017, SDZ-RTX was approved by the EMA for use in all indications included in the label for the EU-authorized reference medicine, including NHL (FL and DLBCL), CLL, RA, GPA, and MPA [14]. SDZ-RTX has since then received regulatory approval in highly regulated markets, including Switzerland [35], Japan, Australia [36], and New Zealand, as well as other territories, for all indications of reference rituximab based on the extrapolation approach (Table 3).

Conclusion

Analytical data demonstrate that SDZ-RTX has an identical amino acid structure and comparable physicochemical and functional properties to reference rituximab, with clinical trials in patients with advanced FL or RA confirming that the two medicines have comparable PK, PD, efficacy, safety, and immunogenicity profiles. Based on the totality of evidence for biosimilarity, and existing knowledge of the mechanism of action in each approved clinical indication, healthcare providers and patients can expect the same clinical outcomes with SDZ-RTX and reference medicine across the same indications of use. The introduction of a biosimilar rituximab provides a potential means of providing high-quality, effective, affordable treatment to larger numbers of patients with hematological malignancies and RA, GPA, and MPA than are currently able to access this important medicine.

Future perspectives

As more original biological medicines reach the end of their patent protection periods, new biosimilars are likely to become available as alternative treatment options. Increased availability and familiarity with biosimilar concepts should lead to wider uptake of biosimilar medicines. Since biosimilars are available at lower costs than original biological medicines, increasing the use of biosimilars will help to reduce healthcare costs, not only enabling wider access to current therapies but also enabling access to higher cost novel treatments. In addition, savings resulting from the use of biosimilar medicines could be invested in innovation of novel therapeutics. The rituximab biosimilar SDZ-RTX (Rixathon®) has recently gained approval in the EU for treatment of non-Hodgkin's lymphomas

Table 3. Summary of primary and key secondary PK/PD results (PK analysis set) in a study involving patients with rheumatoid arthritis treated with SDZ-RTX or reference rituximab.

PK parameter	Treatment	n	Adjusted geometric mean	Treatment comparison		
				Drugs	Geometric mean ratio	90% CI
Primary PK endpoints						
AUC _{0-inf} (μg/ml·24h)	SDZ-RTX	124	7627	RTX-US vs RTX-EU	1.09	(0.99–1.21)
	RTX-US	80	7537	SDZ-RTX vs RTX-US	1.01	(0.93–1.11)
	RTX-EU	79	6897	SDZ-RTX vs RTX-EU	1.11	(1.01–1.21)
Key secondary PK endpoints						
C _{max} 1 st infusion (μg/ml)	SDZ-RTX	120	362	RTX-US vs RTX-EU	1.05	(0.95–1.17)
	RTX-US	82	336	SDZ-RTX vs RTX-US	1.08	(0.98–1.18)
	RTX-EU	78	320	SDZ-RTX vs RTX-EU	1.13	(1.03–1.24)
Main PD endpoint (B-cell depletion)						
AUEC _{0-14 d} (%·24h)	SDZ-RTX	110	1227	RTX-US vs RTX-EU	1.03	(1.02–1.05)
	RTX-US	80	1241	SDZ-RTX vs RTX-US	0.99	(0.97–1.00)
	RTX-EU	76	1201	SDZ-RTX vs RTX-EU	1.02	(1.00–1.04)
AUC: Area under the plasma concentration time curve; AUEC: Area under the effect time curve; C _{max} : Maximum plasma concentration; PD: Pharmacodynamic; PK: Pharmacokinetic; RTX: Rituximab. Created using data from [30].						

AUC: Area under the plasma concentration time curve; AUEC: Area under the effect time curve; C_{max}: Maximum plasma concentration; PD: Pharmacodynamic; PK: Pharmacokinetic; RTX: Rituximab.

Created using data from [30].

(follicular lymphoma and diffuse large B-cell lymphoma), chronic lymphocytic leukemia, rheumatoid arthritis, granulomatosis with polyangiitis, and microscopic polyangiitis. Approval was based on a comprehensive assessment of comparability with reference rituximab, including analytical, preclinical, and selective clinical data. SDZ-RTX offers a significant cost-saving potential compared with reference rituximab, and this may allow a broader population of patients to access this beneficial therapy, particularly in countries with financially constrained healthcare systems. In countries where access to biologics is more widespread, the use of biosimilar rituximab may free up resources to allow healthcare systems to provide increased access to more expensive and innovative new therapies.

Supplementary data

An infographic accompanies this paper at the end of the references section. To download the infographic that accompanies this paper, please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/fon-2019-0430

Author contributions

All authors provided substantial contributions to the conception or design of the work, drafted the work or revised it critically for important intellectual content, provided final approval of the version to be published, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgments

I Feuerstein of Sandoz Biopharmaceuticals provided a significant contribution to reviewing the manuscript for important intellectual content. E Sasse, O Harlin and SO Mueller formerly of Sandoz Biopharmaceuticals, read and reviewed the manuscript.

Financial and competing interests disclosure

S Cohen is on a Sandoz rituximab advisory panel funded by Sandoz. W Jurczak and TM Illidge are on a Sandoz rituximab advisory panel funded by Sandoz and were clinical investigators in clinical trials with Sandoz rituximab. J Amersdorffer is an employee of Sandoz. A da Silva was formerly an employee of Sandoz and is now an independent consultant. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Information pertaining to writing assistance: Editorial assistance in the form of medical writing services was provided by S Clissold, R Furlong and P Weber of Content Ed Net, Germany, and B Caldwell and A Hatton of Spirit, a division of Spirit Medical Communications Group Limited, Manchester, UK. This assistance was funded by Sandoz, a Novartis division.

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Executive summary

Background

- For regulatory approval, a biosimilar agent must undergo a comprehensive stepwise comparison with its reference medicine to provide clear evidence of biosimilarity. The comparability exercise includes generation of comparative data for physicochemical, structural, functional and biological attributes, followed by *in vivo* preclinical studies in case of any residual uncertainty. Finally, clinical studies of pharmacokinetics/pharmacodynamics (PK/PD), safety (including immunogenicity) and efficacy are performed.

Rituximab

- Rituximab is a CD20-targeting antibody that acts through B-cell depletion and is approved in a number of indications in oncology and immunology, including non-Hodgkin's lymphomas (NHL: follicular lymphoma [FL] and diffuse large B-cell lymphoma [DLBCL]), chronic lymphocytic leukemia, rheumatoid arthritis (RA), granulomatosis with polyangiitis, and microscopic polyangiitis.
- Antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) are thought to be the key effector functionalities that contribute to rituximab-mediated B-cell depletion.

SDZ-RTX: a rituximab biosimilar

- Sandoz rituximab (SDZ-RTX; Rixathon®), a rituximab biosimilar, was approved in the EU in June 2017 for treatment of all approved indications of reference rituximab, followed by approval in many other territories globally.
- The development of SDZ-RTX followed the EMA- and US FDA-recommended stepwise approach, involving target-directed development, comprehensive physicochemical and biological characterization and preclinical evaluations, and clinical testing in suitably sensitive indications.

SDZ-RTX development

- The initial step in the development process involved a comprehensive analysis of the quality attributes of reference rituximab, to allow iterative target-directed development of the biosimilar to match the reference medicine.
- This was followed by extensive structural and physicochemical analysis to ensure matching properties with regard to: amino acid sequence; primary, secondary and higher order protein structure; and post-translational modifications.
- The next stage was to establish a close match of SDZ-RTX to the functional and biological characteristics of reference rituximab. SDZ-RTX demonstrated equivalence with reference rituximab in terms of receptor binding characteristics, B-cell depletion properties and functional activity.
- Preclinical *in vivo* characterization included evaluations of toxicity, immunogenicity, and PK/PD in animal studies. For SDZ-RTX, PK/PD analyses in cynomolgus monkeys and evaluation of inhibition of tumor growth in two xenograft mouse models of NHL demonstrated comparable results for SDZ-RTX and reference rituximab.
- The final development step for SDZ-RTX was evaluation of safety and efficacy in sensitive indications – that is, disease settings in which detection of clinically relevant differences between the two medicines would be most likely, should any differences be present.
- Clinical studies comprised a Phase II trial in patients with RA, and a Phase III, confirmatory efficacy trial in patients with FL. Data showed that SDZ-RTX has comparable PK, PD (B-cell depletion), efficacy, safety, and immunogenicity to reference rituximab in both of these indications.
- Overall, the totality of evidence from analytical, preclinical, and clinical studies demonstrates that SDZ-RTX matches reference rituximab across all clinically relevant domains, including structural, physicochemical, functional and biological properties, quality, efficacy, and safety and tolerability (including immunogenicity).
- These data, along with existing knowledge that the mechanism of action of rituximab is shared across each approved clinical indication, formed the basis for approval of SDZ-RTX in all indications of reference rituximab via the principle of extrapolation of data with the reference medicine to other approved indications not tested with SDZ-RTX.

Conclusion

- Based on the extensive, rigorous stepwise comparability exercise conducted to establish biosimilarity of SDZ-RTX with reference rituximab, healthcare providers and patients can expect the same clinical outcomes with SDZ-RTX and reference rituximab across the same indications of use.

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Sandoz rituximab: foundations of development

What is a biosimilar?

A biologic medicine that **matches** an existing biologic medicine¹

The focus of biosimilar development is different from that of an original biologic

- the focus is **not to establish patient benefit per se**, as that has already been performed for the original biologic (the reference medicine)²
- the aim is to conclusively demonstrate similarity by **removing any uncertainty** regarding potential clinical differences between the biosimilar and the reference medicine.³

Demonstration of similarity is achieved by a stepwise development program, with each step reducing the residual uncertainty regarding potential clinical differences.³ The evidence generated by all steps together is known as the **totality of evidence for biosimilarity**³

Regulatory agencies agree that the initial step in biosimilar development should be an extensive analytical comparability exercise focusing on demonstration of matching structural, physicochemical and biological COAs. Demonstrating a close match at this fundamental level reduces the extent of animal and clinical testing required.^{4,5}

If the molecules are sufficiently similar, they will behave in the same way

Sandoz rituximab (SDZ-RTX) is an approved biosimilar of rituximab⁶

All COAs for SDZ-RTX were found to be within ranges defined by reference rituximab^{7,8}

SDZ-RTX
REFERENCE RITUXIMAB

Additional testing to confirm biosimilarity

After confirmation of analytical similarity, no scientific reason exists for differences in other parameters³

However, additional testing allows removal of any residual uncertainty – these additional tests are known as **confirmatory tests** or trials, as they **confirm** that the **biosimilar and reference medicine behave similarly** in animals and humans⁹

Confirmatory tests measure:

SDZ-RTX demonstrated similarity to reference rituximab across the full range of analytical, preclinical and clinical assessments^{7,8,10,11}

	Functional testing to confirm mechanism of action ⁷			Biological testing of activity ⁸		Animal and human studies to confirm PK/PD ^{10,11}	Clinical studies to confirm efficacy in whichever indication is most sensitive to detect differences between biosimilar and reference medicine ^{10,11}
	Key effector mechanisms (ADCC, CDC, and apoptosis)	CD20 (target cell) binding	Fc _γ receptor (effector cell) binding	B cell depletion	Tumour growth inhibition		
Reference rituximab							
SDZ-RTX							

MATCH MATCH MATCH MATCH MATCH

SDZ-RTX demonstrated equivalent PK and PD to reference rituximab

SDZ-RTX demonstrated equivalent immunogenicity, safety and efficacy to reference rituximab in both sensitive indications

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