

**A RANDOMIZED, MULTIPLE-DOSE, MULTICENTER, COMPARATIVE PARALLEL STUDY TO EVALUATE THE PHARMACOKINETIC CHARACTERISTICS OF INTRAVENOUS INFUSION OF RITUXIMAB (HETERO) AND REFERENCE MEDICINAL PRODUCT (RITUXIMAB, ROCHE) IN INDIAN PATIENTS OF NON-HODGKIN'S LYMPHOMA****Dr Shubhadeep Sinha\*, Mr Bala Reddy, Mr Vamsi Krishna Bandi, Dr Pankaj Thakur\*, Dr Neetu Naidu and Dr Sreenivasa Chary**

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**ABSTRACT**

**Aim:** To evaluate the pharmacokinetic characteristics of two formulations of Rituximab (test and reference) at 1<sup>st</sup> & 6<sup>th</sup> cycles (steady state) of chemotherapy. **Methods:** A total of 135 Diffuse Large B-cell Lymphoma (DLBCL) patients were randomized to receive intravenous infusion of either test or reference product. Pharmacokinetic assessment was done for 50 DLBCL patients. Serial PK samples were collected during cycle one (pre and post infusion) and cycle six (pre and post infusion) of chemotherapy.  $C_{max}$ ,  $T_{max}$ ,  $AUC_{0-t}$ , and  $AUC_{0-22d}$ , were assessed for both the cycles. **Results:** Values of  $C_{max}$  (ng/ml) for test  $322572.416 \pm 112718.8714$  and reference  $294696.340 \pm 83273.4206$ ,  $T_{max}$  (hours) for test 4.583 (2.000 - 507.600) and reference 4.350 (2.000-507.933),  $AUC_{0-t}$ , and  $AUC_{0-22d}$  were comparable for both cycle one after single dose administration and at steady state in cycle six of test product and reference medicinal product in DLBCL patients. **Conclusion:** The pharmacokinetic parameters of both test and reference products of Rituximab were found to be comparable in patients of Non-Hodgkin's lymphomas (viz; DLBCL) treated with R-CHOP.

**KEYWORDS:** Rituximab, Non-Hodgkin's Lymphoma, Follicular Lymphoma, Diffuse Large B-Cell Lymphoma.**INTRODUCTION**

Diffuse large B-cell lymphoma (DLBCL) is an aggressive type of non-Hodgkin lymphoma that develops from the B-cells in the lymphatic system. The gold standard for its treatment has been CHOP (cyclophosphamide, doxorubicin, prednisone and vincristine) regimen for more than twenty years.<sup>[1-3]</sup> After the introduction of Rituximab (a chimeric antiCD20 human immunoglobulin G1 monoclonal antibody) in 1998 many studies demonstrated the survival advantage with adding Rituximab to CHOP regimen.<sup>[2,4,5]</sup> Consequently, R-CHOP has now become the standard of care for the treatment of CD20-positive B-cell lymphomas. Rituximab had received formal FDA regulatory approvals for treatment of non-Hodgkin lymphoma and chronic lymphocytic leukemia and, in combination with methotrexate, as second-line therapy for adult patients with moderate to severe rheumatoid arthritis. Rituximab received its initial FDA approval for marketing in the USA in 1997 and in the European Union in 1998.<sup>[6,7]</sup>

This product also got approved in India, however, in India, cost remains a major limiting factor for

widespread use of Rituximab. Therefore, an introduction of biosimilar was sought to be helpful for bridging the gap. A biosimilar product is a biological product that is approved based on a showing that it is highly similar to an FDA-approved biological product, known as a reference product, and has no clinically meaningful differences in terms of safety and effectiveness from the reference product<sup>[8]</sup> or biosimilars are the generic equivalents of innovator biologics.<sup>[9]</sup> Production and manufacturing of biosimilars is a relatively complex method and involves separate steps before final approval.<sup>[10]</sup> In India, safety of biosimilar rituximab is already established in Indian patients with rituximab biosimilar products, available in Indian market since 2012. However, the approval of these products were based on a phase-3 safety and efficacy study in Indian patients of DLBCL variant of non-hodgkin's lymphoma (NHL). A pharmacokinetic comparison between a biosimilar of rituximab and innovator product in Indian patients have not been reported. Hence this study was designed to compare the pharmacokinetics of intravenous infusion of test and reference medicinal products in patients of non-hodgkin's lymphoma

(DLBCL) and the study results are presented in this article.

## METHODS

### 1. Investigational and Reference Medicinal Products

As an investigational medicinal product, Rituximab (Hetero) (T) for intravenous infusion, each single use 50 ml and 10 ml single use vial containing 500 mg and 100 mg of Rituximab respectively were administered.

As a reference medicinal product, Rituximab (Roche) (R), for intravenous infusion each 50 ml and 10 ml single use vial containing 500 mg and 100 mg of Rituximab were administered.

### 2. Study design

This was a phase 3, randomized, multiple-dose, multi-center, comparative parallel study to evaluate the efficacy, safety of intravenous infusion of Rituximab, test and reference medicinal product in Indian patients of newly diagnosed Diffuse Large B Cell Non-Hodgkin's Lymphoma (DLBCL)... The study was carried out from Sep 2013 to Aug 2015 at 36 sites of India. The study protocol was approved by office of Drug Controller General of India (DCGI) and Ethics Committees. Independent ethics committees or institutional review boards at participating sites approved the protocol. The study was registered at clinical trial registry-India (CTRI) prior to initiation of the study (CTRI Registration No: CTRI/2013/08/003921). The study was conducted in accordance with the Declaration of Helsinki (2000), applicable local regulatory guidelines and the International Conference on Harmonisation Guidelines for Good Clinical Practice (ICH-GCP). Written informed consent was obtained from patients or their legally authorized representatives before initiation of any trial procedures.

Key inclusion criteria were male or female  $\geq 18$  years and  $\leq 65$  years of age (both inclusive), histologically confirmed CD20-positive, newly diagnosed diffuse large B-cell Non-Hodgkin's lymphoma (Stage I, II, III, IV by the Ann Arbor (Cotswold modification) in combination with chemotherapy, patients who are eligible for Rituximab and CHOP, patients with at least one measurable lesion as per International Working Group Response (IWGR) criteria for malignant lymphoma, adequate liver, renal, cardiac and hematological function, subjects with a performance status of 0 to 2 according to the Eastern Cooperative Oncology Group (ECOG), life expectancy more than six months.

135 patients of DLBCL were randomized to receive either intravenous infusion of Hetero-Rituximab or reference medicinal product (Rituximab, Roche) at a ratio of 1:1 to have 104 evaluable patients. From these enrolled patients, 50 patients (25 in each arm) were included for pharmacokinetic measurements after cycle 1 and cycle 6 dosings.

## Pharmacokinetic Sampling Schedule

### Cycle 1, Day 1

Serial PK blood samples were collected pre-infusion, 2 hours ( $\pm 10$  minutes) and 3 hours ( $\pm 10$  minutes) after the start of infusion, at the end of infusion (a 10-minute window from the end of infusion was permissible) and at 0.5-hour ( $\pm 10$  minutes), 1 hour ( $\pm 10$  minutes) and 6-hour ( $\pm 10$  minutes) post infusion.

### Cycle 1, Days 2 to 22

Serial PK samples were obtained at 24, 48, and 72 hours after the end of infusion on Days 2, 3, and 4, respectively, allowing a window of  $\pm 1$  hour at each time point. Samples then collected on Days 8, 15 and 22, allowed a window of  $\pm 4$  hours at each time point. The Day 22 sample obtained prior to the initiation of second cycle Rituximab dosing. Day 22 was the conclusion of PK sampling for Cycle 1 regardless of whether the second cycle commenced on Day 22.

PK assessment was done with approximately 31.5 ml of blood sample per patient (12 samples of 2 ml each and 1 pre-dose samples of 4 ml each+07 samples of 0.5 ml each of discarded heparinised saline blood) at cycle 1.

### Cycle 6, Day 1

Serial PK blood samples were collected pre-infusion, 2 hours ( $\pm 10$  minutes) and 3 hours ( $\pm 10$  minutes) after the start of infusion, at the end of infusion (a 10 minute window from the end of infusion is permissible) and at 0.5 hour ( $\pm 10$  minutes), 1 hour ( $\pm 10$  minutes) and 6 hours ( $\pm 10$  minutes) post infusion.

### Cycle 6, Days 2-22

Serial PK samples were obtained at 24, 48 and 72 hours after the end of infusion on Days 2, 3 and 4, respectively allowing a window of  $\pm 1$  hour at each time point. Samples were collected on Days 8, 15 and 22 allowing a window of  $\pm 4$  hours at each time point. Pharmacokinetic (PK) set was made from Pharmacokinetic population, defined as patients who provide PK samples for the required evaluations.

## Assessment of Pharmacokinetic Parameters

For the pharmacokinetic evaluations, a total of 13 blood samples were collected in cycle 1 and cycle 6 at the time points specified in the protocol. Pharmacokinetic analyses i.e.  $C_{max}$ ,  $T_{max}$ ,  $AUC_{0-t}$ ,  $AUC_{0-22d}$  for cycle 1 and  $C_{max,ss}$ ,  $T_{max,ss}$ ,  $AUC_{0-\tau,ss}$ ,  $C_{min,ss}$ ,  $C_{av,ss}$ , %Fluctuation, and %Swing for cycle 6 were performed on the pharmacokinetics set, consisting of all randomized patients who receive at least 1 dose of study drug and have sufficient concentration data to characterize the pharmacokinetic parameters. A non-compartmental model 202 using WinNonlin Professional Software Version 5.3 (Pharsight Corporation, USA) was used to calculate PK parameters from the drug concentration-time profile. Descriptive statistics was computed and reported for pharmacokinetic parameters of Rituximab at Cycle 1 and 6.

These PK parameters were calculated using actual time of sample collection at cycle 1 and using scheduled (i.e. nominal) time points at cycle 6. Actual infusion rate was considered for pharmacokinetic analysis for both the cycles. All concentration values below the lower limit of quantification were set to zero for the pharmacokinetic and statistical calculations. Any missing samples (M) and/or non-reportable (NR) concentration values were not considered for analysis. The overall summary of PK parameters for cycle 1 and cycle 6 along with descriptive statistics for reference and test product are given in Table 1 and table 2 respectively.

## RESULTS

All the 50 patients who gave consent for Pk measurements were included in PK analysis at cycle 1 and at cycle 6, only 43 pk consented patients were included (7 patients were lost to follow-up at after cycle 1). Plasma PK concentrations are shown in Figure 1 for cycle 1 for both test and reference. Non-compartmental parameters for cycle 1 and 6 are provided in Table 1 and 2 respectively. Absorption was comparable between test and reference after cycle 1 and cycle 6.

- After cycle 1, the median Tmax of for test and reference products were 4.58 hrs and 4.35 hrs respectively. After cycle 6 dosing as well. Tmax was comparable between test and reference product with a median value of 3.409 (2.000 - 9.283) and 3.667 (2.000 - 51.700) respectively.
- AUC<sub>0-t</sub> for test and reference products after cycle 1 were 41234643.186 ± 20624906.7064 ng.h/ml and 37864183.358 ± 13269431.1459 ng.h/ml respectively. AUC<sub>0-22d</sub> for cycle 1 for test and reference products were 41046441.296 ± 20428826.4097 ng.h/ml and 37871913.745 ± 12699562.9775 ng.h/ml respectively.
- AUC<sub>0-τ,ss</sub> after cycle 6 were 58604452.352 ± 17396817.9462 ng.h/ml and 60008796.985 ± 15966838.2060 for test and reference products respectively.
- Maximum observed plasma concentration (C<sub>max</sub>) after cycle 1 were 322572.416 ± 112718.8714 ng/ml and 294696.340 ± 83273.4206 ng/ml for test and reference products respectively. For cycle 6 C<sub>max,ss</sub> was 318804.236 ± 67022.2616 ng/ml and 314477.319 ± 71926.2133 ng/ml for test and reference products respectively.

**Table: 1 Descriptive Statistics of Formulation Means for Rituximab in Cycle 1**

Parameters (Units)	Mean ± SD	
	Test Product (N = 25)	Reference Product (N = 25)
T <sub>max</sub> (h) <sup>#</sup>	4.583 (2.000 - 507.600)	4.350 (2.000-507.933)
C <sub>max</sub> (ng/mL)	322572.416 ± 112718.8714	294696.340 ± 83273.4206
AUC <sub>0-t</sub> (ng.h/mL)	41234643.186 ± 20624906.7064	37864183.358 ± 13269431.1459
AUC <sub>0-22d</sub> (ng.h/mL)	41046441.296 ± 20428826.4097	37871913.745 ± 12699562.9775

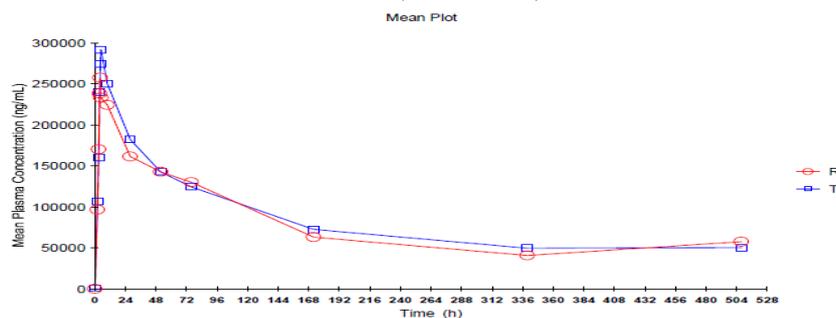
<sup>#</sup>T<sub>max</sub> is represented as median (min-max) value

**Table: 2 Descriptive Statistics of Formulation Means for Rituximab in Cycle 6**

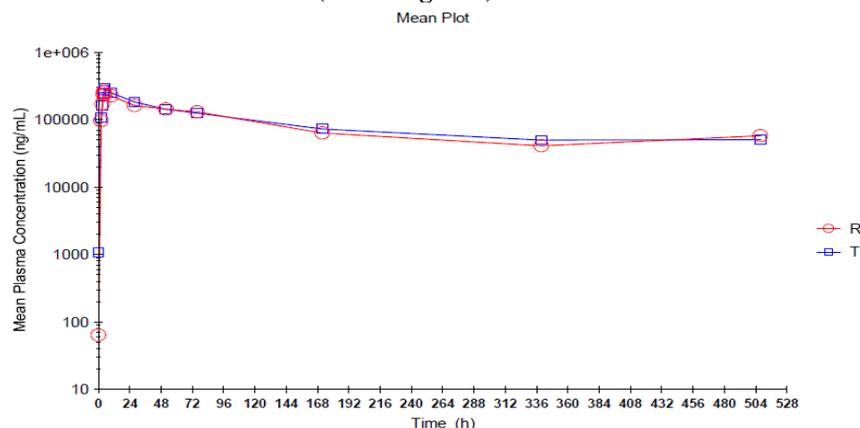
Parameters (Units)	Mean ± SD	
	Test Product (N = 22)	Reference Product (N = 21)
T <sub>max,ss</sub> (h) <sup>#</sup>	3.409 (2.000 - 9.283)	3.667 (2.000 - 51.700)
C <sub>max,ss</sub> (ng/mL)	318804.236 ± 67022.2616	314477.319 ± 71926.2133
AUC <sub>0-τ,ss</sub> (ng.h/mL)	58604452.352 ± 17396817.9462	60008796.985 ± 15966838.2060
C <sub>min,ss</sub> (ng/mL)	59540.727 ± 28059.4464	57050.148 ± 19116.0273
C <sub>av,ss</sub> (ng/mL)	116278.675 ± 34517.4959	119065.073 ± 31680.2345
%Fluctuation	243.829 ± 109.5189	226.088 ± 63.7351
%Swing	784.987 ± 1055.6350	510.273 ± 260.5960

<sup>#</sup>T<sub>max</sub> is represented as median (min-max) value.

**Figures: Mean Plasma Concentration Vs Time Curve (Linear Plot)**



**Figure: 1 Mean Plasma Concentration vs. Time Curve for Rituximab in Cycle 1 (N = 25 for Test Product and N = 25 for Reference Product) [Upper Panel: Linear Plot,**

**Figures: Mean Concentration Vs Time Curve (Semi Log Plot)**

**Figure: 2 Mean Plasma Concentration vs. Time Curve for Rituximab in Cycle 1 (N = 25 for Test Product and N = 25 for Reference Product) Semi-logarithmic Plot]**

**DISCUSSION**

Rituximab has been the mainstay in treatment of non-Hodgkin's lymphoma chronic lymphocytic leukemia and rheumatoid arthritis after its approval by FDA as the first monoclonal antibody.<sup>[11]</sup> Despite its popularity in India, its cost was the limiting factor in its widespread use. Roy et al had compared toxicity, tumor response rates, Progression Free Survival (PFS) and Overall Survival (OS) in a retrospective study but it did not comprise pharmacokinetic properties of MabThera™ and an Indian biosimilar of rituximab.<sup>[12]</sup>

In this study, approach. In this study, the pharmacokinetic parameters were comparable after single dose administration (cycle 1) of test product and reference medicinal product in DLBCL patients.

The pharmacokinetic parameters of test Product and reference medicinal products were also comparable at steady state (Cycle 6) in DLBCL patients.

Several studies have analyzed pharmacokinetics (PK) of Rituximab in patients receiving treatment of active, relapsed, or refractory NHL. Results of these studies indicate that serum Rituximab levels are highly variable in patients receiving similar doses. The results observed in our study are comparable to other published studies of Mabthera™ which used a population pharmacokinetic analysis.<sup>[13-17]</sup>

In the case of follow-on biologics, comparability of manufacturing process, comparable pharmacokinetics as well as efficacy responses are an indicator of therapeutic equivalence to prove equivalence and interchangeability. In this study, the comparability of pharmacokinetic parameters was observed, indicating comparability of Hetero-Rituximab with the innovator.

The innovator rituximab was not affordable and available to a large majority of patients in a developing country like India where health insurance schemes have limited implications. Hence introduction of another biosimilar of

Rituximab, Hetero-Rituximab (MabAll, Rilast) is likely to improve access to Rituximab and might be used interchangeably with reference Rituximab.

**CONCLUSION**

Test Rituximab (Hetero) was found pharmacokinetically comparable with Rituximab, Roche for the treatment of B-cell lymphomas.

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