NON-INTERVENTIONAL STATISTICAL ANALYSIS PLAN FOR SECONDARY DATA COLLECTION STUDY

B1931043

A retrospective analysis of Inotuzumab ozogamicin (InO) usage in adult patients with relapsed/refractory (R/R) B-cell Acute Lymphoblastic leukemia (ALL)

Statistical Analysis Plan
(SAP)

Version: 1.0

Author: PPD

Date: 04-May-2023

Author: PPD

Date: 04-May-2023

TABLE OF CONTENTS

1 AMENDMENTS FROM PREVIOUS VERSION(S)

None

2 INTRODUCTION

B-cell acute lymphoblastic leukemia (ALL) is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood,and extramedullary sites. Only 20% of ALL occurs in adult patients.[i] Incidence data on adult B-cell ALL in India is lacking.[ii] The regimens used for pediatric patients with B-cell ALL are mostly used for the treatment of adult patients with B-cell ALL. The treatment of B-cell ALL in adults is mostly based on regimens developed for pediatric patients.

Most chemotherapy regimens used to treat adults with B-cell ALL in India consists of multi-agent induction, intensification or consolidation, and maintenance phases, together with central nervous system (CNS) prophylaxis using intrathecal chemotherapy, with or without cranial irradiation. Commonly used cytotoxic agents in first line treatment protocol for adult patients with B-cell ALL are cyclophosphamide, vincristine, daunorubicin, L-asparaginase and prednisolone. The prognosis of R/R B-cell ALL has historically been dismal with complete remission (CR) rates of 20-40%, median Overall Survival (OS) of 6 months, and cure rates of < 10% even with intensive salvage chemotherapy and haematopoietic stem cell transplantation.[iii]

Relapsed/Refractory B-cell ALL in adults is a challenging disease, with high mortality resulting from refractory disease or toxicity from intensive chemotherapy regimens. The only curative option in this subset of patients is CR after salvage therapy followed by Allogeneic hematopoietic cell transplantation (Allo-HCT). Even salvage regimens in case of relapse are mostly composed of old agents used in first-line protocols. Although second remissions are possible, with these salvage regimes, these are of short duration unless an allogeneic stem cell transplant is feasible.[iv] The data on the outcomes of salvage therapy in adult patients with B-cell ALL is lacking from India. However, novel therapies such as Ino have improved the outcomes of this subset of patients, with more patients experiencing CR and minimal residual disease negativity, thus becoming eligible for allo-HCT. The real-world data on the use of Ino monotherapy in cases of adult R/R B cell ALL is limited in Indian settings with knowledge gaps relating to diagnostic and treatment practices. Currently, only a single study is available with Cancer of unknown primary (CUP) data from 8 patients.

InO is an antibody-drug conjugate consisting of a recombinant, humanized IgG4 antibody (G544) covalently bound to a semi synthetic derivative of calicheamicin. Calicheamicin, a cytotoxic natural product produced by microbial fermentation that is significantly more potent than conventional cytotoxic chemotherapeutic agents, binds to the DNA minor groove and causes cell death by inducing double strand DNA breaks. The targeting agent in InO, (G544)

specifically recognizes human CD22, which is expressed on the malignant cells of the majority of B-lymphocyte malignancies, including on the surface of blasts in >90% of patients with B cell ALL.[v] *In 2017, the United States (US) Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved InO for the treatment of adult with R/R B-cell precursor B-cell ALL.*[vi,vii]

Inotuzumab: mode of action and literature evidence

ALL is the second most common acute leukemia in adults. The characteristic features are chromosomal abnormalities and genetic alterations involved in differentiation and proliferation of lymphoid precursor cells. The backbone of therapy remains multi-agent chemotherapy with vincristine, corticosteroids and an anthracycline with allogeneic stem cell transplantation for eligible candidates.[viii]

The advent of monoclonal antibody (moAb) targeting CD22 (InO) and CD19 (blinatumomab), have dramatically improved the outcome of adults with R/R B cell ALL. These have shown significant survival benefits against standard therapies and expanded the armamentarium of ALL.[ix]

InO is an anti-CD22 moAb conjugated to the cytotoxic antibiotic calicheamicin. Based on promising phase I/II data, InO was compared to standard salvage chemotherapy in Phase 3 multicenter trial rINO-VATE) of 218 adult patients with CD22+ B cell ALL. The overall response and Minimal residual disease (MRD) negativity rates among responders were significantly higher with InO compared with chemotherapy (81% versus 29%, $p < 0.001$, and 78% versus 28%, $p < 0.001$, respectively). More patients who received InO were able to undergo HSCT (41% versus 11%; $p < 0.001$). The median remission duration and progression-free survival were significantly longer with InO (4.6 versus 3.1 months; $p = 0.03$, and 5.0 versus 1.8 months; $p < 0.001$, respectively). The median OS was 7.7 versus 6.7 months ($p = 0.04$). This was later confirmed with longer follow-up on 326 patients showing 2-year OS rates of 23% versus 10% ($p = 0.01$) in favor of InO [24]. Predictors for better survival included achievement of CR, MRD negativity, and consolidative HSCT. Patients who achieved MRD negativity derived more benefits regardless of the number of prior therapies.[x]

InO is an antibody drug conjugates (ADC) comprising of a moAb targeting CD22,[xi] *a cell surface antigen expressed on approximately 90% of B-cell malignancies,*[xii] *linked to a cytotoxic agent.*

CD22 is an important modulator of B-cell lymphocyte function and survival,[xiii] *and is expressed on mature B-cells, which may allow for targeted delivery of the cytotoxic agent.*[xiv]

When InO binds to the CD22 antigen on malignant B-cells, it is absorbed into the cell, at which point the cytotoxic agent calicheamicin is released to destroy the cell.[xv]

Adding an ADC targeting CD22, such as InO, to existing treatments options, may provide additional anti-tumor activity.[xvi]

2.1 STUDY DESIGN

The study is classified as a Non-interventional study (NIS) secondary data collection-unstructured review with sites/investigators.
R/R B-cell ALL patients who are ≥18 years old at the time of InO initiation and those who received the drug outside of clinical trials will be evaluated.

Data will be collected retrospectively from patients hospital medical records (paper-based) by members of the direct care team (investigators). We intend to have 7- 10 sites across India using InO monotherapy including patients with both private purchase and compassionate use to reach a cohort of approximately 55 patients. If needed, more sites will be enrolled to meet the target cohort. Data will be reviewed and analyzed from all the sites.

Medical records will be reviewed to collect demographic, patient-related, disease-related, and clinical outcome data. These patients will be evaluated for response (CR/CRi), MRD status, duration of remission (DOR), dosage patterns, rate of transplant, survival time for all patients including survival rate at 6 months and 12 months in transplanted patients & non transplanted patients, Relapse free survival (RFS) time, Rate of veno-occlusive disease (VOD) (total during study treatment and post HSCT if any patient underwent HSCT), safety/tolerability, Other adverse effects (including hematological toxicities and raised liver enzymes), Number of cycles to achieve MRD negativity in responders & CR/CRi in Extramedullary disease / lymphoblastic lymphoma (EMD/LBL).

Study population

This is a descriptive study and there is no prior hypothesis specified, therefore a formal sample size calculation is not required. The sample size of approximately 55 patients has been based on the number of patients expected to be available at these 7-10 sites that individually treated the largest number of patients with InO.
The study will include information on approximately 55 patients as per the availability of data from 7-10 sites.

Data source

Data for this study will be collected through retrospective data collection from 7-10 sites for patients in India who received Inotuzumab for the indication of adult R/R B Cell ALL between

the time period Feb'2017 – Feb'2022. Additional centers may be added at a later stage if the target sample size is not achieved.
Data will be collected in anonymized form on Paper/excel based CRF for the study. Participants will be identified in all study records by a unique participant identification number to allow data management queries to be resolved with reference to source medical records while preserving patient confidentiality.
Medical records will be reviewed from secondary data sources to collect demographic, patient-related, disease-related, and clinical outcome data & Adverse Event's. These patients will be evaluated for response, duration of response (DOR), survival time from the time of InO initiation till the death or the time data will be retrieved.
Data to be verified in accordance with protocol standards. Before obtaining the data from the selected sites across the nation, the necessary regulatory approval will be ensured.

Treatment/cohort labels

The study group will be R/R B-cell ALL patients who are ≥18 years old and InO monotherapy initiated. As no other group exist, a unique ID will be assigned to each patient who will be included in the study which must consist site number along with their registration number assigned from the site.

2.2 STUDY OBJECTIVES

Aim: We aim to carry out a multicentric retrospective cohort analysis study with data from approximately 55 patients to observe the clinical outcomes among patients treated with InO monotherapy in real-world clinical practice in India.

Primary objective

The primary objective of the study is to assess rate of complete remission or complete remission with incomplete hematological recovery following treatment with InO, by the number of salvage therapies (0, 1, ≥2) received before InO initiation.

Secondary objective

The secondary objective is to explore the presence/occurrence of other factors along with primary outcome, listed as follows

- *MRD Negativity following initiation of Ino*
- *Number of cycles of InO needed to attain CR/CRi*
- *Duration of response(DOR)*
- *Dosage pattern: InO doses, dose modifications, and the number of cycles of treatment*
- *Concomitant medications*

- *Rate of transplant (No of patients who proceeded to transplant)*
- *Survival rate (transplanted patients & non transplanted patients, in both who achieved CR/CRi and MRD negativity)*
- *Cause of death*
- *Relapse-free-survival in all patients and patients with or without follow-up HSCT.*
- *Rate of VOD (Total during study treatment and post HSCT, if any patient underwent HSCT) in transplanted & non transplanted patients.*
- *Occurrence of Grade 3/4 treatment-related adverse events in lung/cardiac/kidney/liver etc following InO initiation; in all patients and patients with or without follow-up HSCT & hematological toxicities*
- *CR/CRi in EMD/LBL*

3 HYPOTHESES AND DECISION RULES

None

3.1 STATISTICAL HYPOTHESES

This is a descriptive study and there is no prior hypothesis specified.The objective of this study is just to observe clinical outcomes among patients treated with InO monotherapy in real-world clinical practice in India.

3.2 STATISTICAL DECISION RULES

As no specified hypothesis will be tested, no decision rule are required to specify. However, for any assessment of external validity for a suitable population accepted false positive rate will be 5%. For any such validation only two sided test will be performed.

4 ANALYSIS SETS/POPULATIONS

The population of interest will be the Relapsed/Refractory B Cell Acute lymphoblastic leukemia adult patients in India who had initiated and completed at least one cycle of InO monotherapy between Feb’2017 and Feb’2022 and CD22 positive.

4.1 FULL ANALYSIS SET

The restrospective available record of all Relapsed/Refractory B Cell Acute lymphoblastic leukemia adult patients satisfying specified inclusion and exclusion criteria will be the full analysis set.

Inclusion criteria

Patients must meet all of the following inclusion criteria to be eligible for inclusion in the study:

- *Patient Population -*
- *Patients aged ≥18 years old at the initiation of InO treatment*
- *Patients with relapsed/refractory B-cell ALL*
- *Patients who initiated InO monotherapy between Feb'2017 and Feb'2022 and are CD22 positive*
- *Ph+ patients who have failed treatment with at least 1 TKI*

Exclusion criteria

- *Patient not completing at least 1 cycle of InO therapy*
- *Patient on InO in combination with chemotherapy*

4.2 SAFETY ANALYSIS SET

- Not required for this study

4.3 OTHER ANALYSIS SET

- No other data set will be used for this study

4.4 SUBGROUPS

The following subgroup will be considered for stratified analysis if data permits with meaningful representation at each subgroup.

- *1st salvage and later lines*
- *High burden and Low burden disease (<50%; 50-90%; >90%)*
- *Ph +ve and Ph – ve patients*
- *Elderly patients> 65 years*

Example
Subgroup analysis may be considered, including but not limited to subgroup analyses by age, dosage, renal impairment, and follow-up length.

5 ENDPOINTS AND COVARIATES

Following local legislation, informed consent won't be signed by the patients since this is a retrospective study and they are not getting enrolled in a prospective clinical trial. All data points mentioned in the inclusion criteria should be available which are necessary to analyze protocol endpoints for the selected patient cohort who has undergone the full treatment of the drug in question. Ethics committee approval of study protocol will be taken to access medical records.

Primary endpoint

Complete remission or complete remission with incomplete hematological recovery following treatment with InO, by the number of salvage therapies (0, 1, ≥2) received before InO initiation.

Secondary endpoints

- *MRD Negativity following initiation of Ino*
- *Number of cycles of InO needed to attain CR/CRi*
- *DOR*
- *Dosage pattern: InO doses, dose modifications, and the number of cycles of treatment*
- *Concomitant medications*
- *Rate of transplant (No of patients who proceeded to transplant)*
- *Survival rate at 6 months & 12 months (transplanted patients & non transplanted patients, in both who achieved CR/CRi and MRD negativity)*
- *Cause of death*
- *Relapse-free-survival in all patients and patients with or without follow-up HSCT.*
- *Rate of VOD (Total, during study treatment and post HSCT, if any patient underwent HSCT) in transplanted & non transplanted patients.*
- *Occurrence of Grade 3/4 treatment-related adverse events in lung/cardiac/kidney/liver etc following InO initiation; in all patients and patients with or without follow-up HSCT & hematological toxicities*
- *CR/CRi in EMD/LBL*

Covariates

- Demographic characteriscs of the patients
- Comorbidities
- Family history

5.1 EFFICACY/EFFECTIVENESS ENDPOINT(S)

The primary outcomes of interest are CR/CRi achieved or not following InO treatment which will be measured by binary response and duration of remission which will be measured by time to achieve CR/Cri from InO therapy starts. Other secondary outcomes will be measured by binary response mostly, but time to event for dealth and relapse will be measured by time from starting of the therapy to the event of interest. The longest time from initiation of InO to date of data extracted will be considered as study period. The number of salvage therapies received prior to the InO therapy initiation will be classified into 0, 1 and >1.

5.2 SAFETY ENDPOINTS

Not applicable for this study

5.3 OTHER ENDPOINTS

None

5.4 COVARIATES

Variable	Role	Data source(s)	Operational definition
Age	Stratified analysis	Patient medical records	
Sex	Stratified analysis	Patient medical records	
Comobidity	Stratified analysis	Patient medical records	Binary response
Family history	Stratified analysis	Patient medical records	Binary response

6 HANDLING OF MISSING VALUES

Owing to the descriptive nature of the study, analysis will be on available data only. No imputation of missing data will be implemented.

7 STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

7.1 STATISTICAL METHODS

Means, medians, and standard deviations will be provided for continuous variables when performing descriptive analysis of continuous data. Numbers and percentages will be provided for dichotomous and polychotomous variables when performing descriptive analysis of categorical data. The proportion of CR/CRi achieved will be compared across the number of salvage therapies prior to InO initiation with respective 95% confidence interval. Kaplan-Meier survival curve will be drawn for longest time from initiation of InO to date of data extracted. Similarly survival curve will be drawn for relaspse free survival. Median survival or restricted mean survival time will be reported across the number of salvage therapies.

7.2 STATISTICAL ANALYSES

The distribution demographics variables like age and sex will be summarized by mean ±SD and frequency (%) respectively. The prevalence of complete remission or complete remission with incomplete hematological recovery following treatment with InO will be reported with 95% confidence interval. Further these prevalence will be estimated for the

number of salvage therapies (0, 1, ≥2) received before InO initiation.6 months or 12 months restricted mean remission free time will be estimated with 95% CI for both complete remission or complete remission with incomplete hematological recovery. The Kaplan-Meier curves of remission free survival will be drawn if a sufficient representation observed across the number of salvage therapies (0, 1, ≥2) received before InO initiation. The restricted mean remission free time with 95% CI will be estimated for categories of number of salvage therapies. Restricted mean/median survival time will also be estimated with 95% CI. The Kaplan-Meier overall survival curve will also be drawn.

Other secondary endpoints like MRD negativity following initiation of InO or dosage pattern, concomitant medications, cause of death , occurrence of Grade 3/4 treatment-related adverse events in lung/cardiac/kidney/liver etc. following InO initiation and CR/CRi in EMD/LBL will also summarized by frequency and percentage and dration of response(DOR), no of patients who proceeded to transplant, total during study treatment and post HSCT will be summarized my median and interquartile range. restricted relapse-free-survival time will also be estimated with 95% CI if data permits for all patients and patients with or without follow-up HSCT.

Due to non-probability sampling technique, the extrapolation of the results will be restricted to the population that matches with sample characteristics only. Magnitude of the measure of any characteristics will be prioritised over statistical interpretation. However, interpretation of the result will be in a qualitative sense even though the related feature is compared numerically owing to descriptive nature of the study.

7.2.1 Safety Analyses

Not applicable

7.2.2 Analyses of endpoint

The following tables and figures will be generated for the analysis of endpoints.

1. The distribution of demographics characteristics (mean ±SD for age and frequency and percentage for sex)
2. MRD negativity following initiation of InO (frequency and percentage)
3. Dosage pattern (frequency and percentage)
4. Cause of death (frequency and percentage)
5. Occurrence of Grade 3/4 treatment-related adverse events in lung/cardiac/kidney/liver etcfollowing InO initiation (frequency and percentage)
6. CR/CRi in EMD/LBL (frequency and percentage)
7. Duration of response (DOR) (Median and Interquartile Range (IQR))
8. No of patients who proceeded to transplant (Median and IQR)
9. Total during study treatment and post HSCT (Median and IQR)
10. Restricted remission free time with 95% confidence interval for both complete remission and complete remission with incomplete hematological recovery.

11. Restricted remission free time with 95% confidence interval for both complete remission and complete remission with incomplete hematological recovery stratified by the number of salvage therapies (0, 1, ≥2) received before InO initiation
12. Restricted mean overall survival time with 95% CI.
13. Restricted relapse-free-survival with 95% CI for all patients and patients with or without follow-up HSCT.

List of figure

1. Kaplan-Meier curve for remission free survival
2. Kaplan-Meier curve for remission free survival stratified by the number of salvage therapies (0, 1, ≥2) received before InO initiation
3. Kaplan-Meier survival curve for overall survival

7.2.3 Summary of Analyses

Not Applicable

8 LIST OF TABLES AND TABLE SHELLS

1. The distribution of demographics characteristics (mean±SD for age and frequency and percentage for sex)
2. MRD Negativity following initiation of InO(frequency and percentage)
3. Dosage pattern(frequency and percentage)
4. Cause of death (frequency and percentage)
5. Occurrence of Grade 3/4 treatment-related adverse events in lung/cardiac/kidney/liver etc following InO initiation (frequency and percentage)
6. CR/CRi in EMD/LBL (frequency and percentage)
7. Duration of response(DOR) (Median and IQR)
8. No of patients who proceeded to transplant (Median and IQR)
9. Total during study treatment and post HSCT (Median and IQR)
10. Restricted remission free time with 95% confidence interval for both complete remission and complete remission with incomplete hematological recovery.
11. Restricted remission free time with 95% confidence interval for both complete remission and complete remission with incomplete hematological recovery stratified by the number of salvage therapies (0, 1, ≥2) received before InO initiation
12. Restricted mean overall survival time with 95% CI.
13. Restricted relapse-free-survival with 95% CI for all patients and patients with or without follow-up HSCT

APPENDICES

Not applicable

8.1 APPENDIX 1: DATA DERIVATION DETAILS

Not applicable

A1.1 Definition and use of visit windows in reporting

None

A1.2 Further definition of endpoints

None

8.2 APPENDIX 2: ADDITIONAL STATISTICAL METHODOLOGY DETAILS

None

A2.1 Further Details of the Statistical Methods

None

8.3 APPENDIX 3: DIAGNOSIS AND PROCEDURE CODES USED IN THE STUDY

None

9 REFERENCES

[i]Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood Cancer J.* 2017;7(6):e577. Published 2017 Jun 30

[ii]Samra B, Jabbour E, Ravandi F, Kantarjian H, Short NJ. Evolving therapy of adult acute lymphoblastic leukemia: state-of-the-art treatment and future directions. *J Hematol Oncol*. 2020;13(1):70.

[iii]Samra B, Jabbour E, Ravandi F, Kantarjian H, Short NJ. Evolving therapy of adult acute lymphoblastic leukemia: state-of-the-art treatment and future directions. J Hematol Oncol. 2020;13(1):70.

[iv]Boni J et al. Modeling the Pharmacokinetic/Pharmacodynamic Platelet Response of Inotuzumab Ozogamicin, a Novel Antibody Drug Conjugate, Administered Alone or in Combination with Rituximab in Patients with Non-Hodgkin's Lymphoma. Accepted Poster Presentation at the European Society of Medical Oncology 2010 Annual Meeting, October 8-12, 2010. Milan, Italy

[v]Leonard J et al. Epratuzumab, a Humanized Anti-CD22 Antibody, in Aggressive Non-Hodgkin's Lymphoma: a Phase I/II Clinical Trial Results. *Clinical Cancer Research.* 2004; 10: 5327-5334.

[vi]Dorner T. Targeting CD22 as a Strategy for treating systemic autoimmune diseases. *Therapeutics and Clinical Risk Management.* 2007; 3: 953-959.

[vii]DiJoseph J et al. Antibody-Targeted Chemotherapy with CMC-544: a CD22 Targeted Immunoconjugate of Calicheamicin for the Treatment of B-Lymphoid Malignancies. *Blood.* 2004; 1-3: 1807- 1814

[viii]Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood Cancer J*. 2017;7(6):e577. Published 2017 Jun 30

[ix]Samra B, Jabbour E, Ravandi F, Kantarjian H, Short NJ. Evolving therapy of adult acute lymphoblastic leukemia: state-of-the-art treatment and future directions. *J Hematol Oncol*. 2020;13(1):70

[x]Samra B, Jabbour E, Ravandi F, Kantarjian H, Short NJ. Evolving therapy of adult acute lymphoblastic leukemia: state-of-the-art treatment and future directions. *J Hematol Oncol*. 2020;13(1):70

[xi]Boni J et al. Modeling the Pharmacokinetic/Pharmacodynamic Platelet Response of Inotuzumab Ozogamicin, a Novel Antibody Drug Conjugate, Administered Alone or in Combination with Rituximab in Patients with Non-Hodgkin's Lymphoma. Accepted Poster Presentation at the European Society of Medical Oncology 2010 Annual Meeting, October 8-12, 2010. Milan, Italy.

[xii]Leonard J et al. Epratuzumab, a Humanized Anti-CD22 Antibody, in Aggressive Non-Hodgkin's Lymphoma: a Phase I/II Clinical Trial Results. Clinical Cancer Research. 2004; 10: 5327-5334.

[xiii]Dorner T. Targeting CD22 as a Strategy for treating systemic autoimmune diseases. *Therapeutics and Clinical Risk Management.* 2007; 3: 953-959

[xiv]DiJoseph J et al. Antibody-Targeted Chemotherapy with CMC-544: a CD22 Targeted Immunoconjugate of Calicheamicin for the Treatment of B-Lymphoid Malignancies. *Blood.* 2004; 1-3: 1807- 1814.

[xv]Boni J et al. Modeling the Pharmacokinetic/Pharmacodynamic Platelet Response of Inotuzumab Ozogamicin, a Novel Antibody Drug Conjugate, Administered Alone or in Combination with Rituximab in Patients with Non-Hodgkin's Lymphoma. Accepted Poster Presentation at the European Society of Medical Oncology 2010 Annual Meeting, October 8-12, 2010. Milan, Italy

[xvi]DiJoseph JF. Antitumor Efficacy of a Combination of CMC-544 (Inotuzumab Ozogamicin), a CD22-Targeted Cytotoxic Immunoconjugate of Calicheamicin, and Rituximab against Non-Hodgkin's B-Cell Lymphoma. Clin Cancer Res. 2006; 12: 242-250