

A Multicenter Asparaginase Monitoring Study in patients with newly diagnosed Acute Lymphoblastic Leukemia (ALL)

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Study Design:

A prospective, multi-centre, observational study to assess the pharmacokinetic characteristics of the pegylated *E.coli* asparaginase biogenerics in newly diagnosed patients with acute lymphoblastic leukemia (ALL) in India during the entire intensive phase of chemotherapy.

Study Drug: Polyethylene glycol (PEG)-conjugated *E. coli* L-Asparaginase

Nodal Study Site: Tata Medical Center, Kolkata, India (TMC)

Participating Study Centres:

1. Government Medical College, Jammu
2. IRCH, All India Institute of Medical Sciences (AIIMS), New Delhi
3. All India Institute of Medical Sciences (AIIMS), Bhubaneswar
4. VMMC and Safdarjung Hospital, New Delhi
5. Homi Bhabha Cancer Hospital and Research Center, Visakhapatnam

Background and rationale:

Asparaginase is a critical component of the multidrug treatment of acute lymphoblastic leukemia (ALL), the most common pediatric cancer worldwide [1]. Every year, approximately 25,000 cases of pediatric ALL are reported from India. ALL is curable with conventional chemotherapy, and over 90% cure rates are achieved in the West with the combination therapy. However, the survival outcomes in India are stagnated at 60-65% due to substantial treatment-related mortality and high relapse rates [1,2].

Polyethylene glycol (PEG)–conjugated formulations of *Escherichia coli* Asparaginases (Ec-ASNase) (Oncaspar, Asparlas) are the standard of care in higher-income countries. Most of the low-middle income countries (LMICs) cannot access these drugs due to its high cost and depends on the biogeneric substitutes marketed in these regions. Unfortunately, the

quality of these biogeneric *E. coli* asparaginases marketed in India is variable [3]. Many generic asparaginases demonstrate shortfalls in critical quality attributes and have variable asparaginase amino acid sequences. These variabilities likely contribute to inferior therapeutic drug activity and higher rates of drug-related hypersensitivity observed with these products [1,4]. Along with native asparaginase products, a number of polyethylene glycol-conjugated (PEG) Ec-ASNases are also available on the market. The mean trough levels of one such Indian PEG-ASNase is lower [5,6], than that reported for the main competitor brand (Oncaspar) [7,8]. For another Indian PEG-ASNase product, trough levels were lower and there was an increase in allergic reactions [9]. To address this problem, we propose establishing an ASNase monitoring program by collaborating with multiple cancer care centers to systematically evaluate their ASNase products. The primary objective is to identify clinically suitable, locally accessible biogeneric ASNases and establish their optimal dosage and administration schedule. This can be accomplished through systematic clinical and pharmacological monitoring within a standardized, risk stratified ALL treatment protocol.

The clinical use of generic native and PEG EcASNases therefore requires systematic pharmacological and clinical monitoring to (a) identify generic products suitable for clinical use and its association with toxicity (b) distinguish between clinical and subclinical hypersensitivity also known as silent inactivation and (c) determine the appropriate dose and dosing schedule of each product.

Primary Objectives:

- To evaluate the pharmacological efficacy of different PEG Asparaginase biogenics administered to patients with ALL across India, determined by therapeutic drug monitoring of plasma asparaginase activity levels serially post each dose of PEG Asparaginase till the end of treatment.

Secondary Objectives:

- To assess the presence of anti-asparaginase antibodies in patients showing silent inactivation.

Study Outcomes:

- Efficacy of Biogeneric Formulations:
Assessment of the pharmacokinetic efficacy of different PEG-asparaginase biogenics by evaluating trough activity levels and capturing instances of silent

inactivation—defined as a trough plasma asparaginase activity level <100 IU/L at Day 14 ± 2 post-dose in the absolute absence of clinical hypersensitivity.

- Pharmacokinetic (PK) Outcome:
Trough Serum Asparaginase Activity: The mean or median asparaginase activity level measured at the trough time-point (Day 14 ± 2 post-dose) during the entire treatment phase.
- Therapeutic drug monitoring platform:
Developing a systematic drug monitoring platform in the participating centers by providing the knowledge base.

Eligibility Criteria for enrollment of patients:

- Patients newly diagnosed with B-cell ALL, T-cell ALL/T-cell Lymphoblastic Lymphoma being treated in ICiCLE-ALL protocol and subjected to receive PEG Asparaginase.
- Age: Between 1 to 18 years

Study duration:

- Induction therapy till the end of delayed intensification for a time frame of 12 months, inclusive of the study analysis.

Sample Size:

- 50 patients in total diagnosed with ALL.

Drug Administration:

- PEG Asparaginase administered according to the institutional protocol (ICiCLE-ALL).

Methodology:

Sample collection and processing:

- At each specified time point, 2-4mL of venous blood will be collected in vials labeled with the specific ID of the patient, and the date and time of collection.
- The date and time of collection are also recorded in the patient's handout sheet by the nursing staff or a patient relative.
- Blood samples are to be processed following the guidelines outlined in study protocol (attached with this proposal).
- Collected samples will be shipped to the central laboratory Tata Translational Cancer Research Centre (TTCRC), TMC for asparaginase activity analysis.

Sampling schedule:

Attached separately

Asparaginase activity assay (to be performed at TTCRC):

The quantification of the enzymatic activity of L-Asparaginase is based on the measurement of substrate turnover at a maximum rate. One unit of activity is defined as the amount of enzyme that releases 1 mmol of ammonia and aspartate from 1 mmol asparagine per minute at 37°C. Asparaginase activity in plasma samples is quantified by incubating the samples with 10mM AHA at 37°C. Asparaginase hydrolyses AHA to aspartic acid and hydroxylamine, which, after condensation with 8-hydroxyquinoline, oxidizes to indooxine and is detected at 710 nm [8,10]. A single four-parametric non-linear regression equation is plotted using standards ranging from 5 to 1250 IU/L, and the activity of each sample is calculated using the curve.

Statistical Analysis:

- Demographic and Baseline Characteristics will be presented as means, medians, standard deviations, interquartile ranges (IQRs), minimums, and maximums for all continuous variables, and as numbers and proportions for the categorical variables.
- To compare pharmacokinetic outcomes (e.g., proportion of patients achieving therapeutic trough levels ≥ 100 IU/L) across different groups, a chi-square test or Fisher's exact test will be utilized for categorical outcomes, and ANOVA or Kruskal-Wallis tests for continuous asparaginase activity levels.

Data Management:

- Data collection and entry

All data generated according to the protocol will be recorded continuously in electronic Case Report Forms (eCRF) in the REDCap database, under a study identification number, unique to each individual patient. To protect participant confidentiality, all required Personally Identifiable Information (PII) and Protected Health Information (PHI) will be fully de-identified at the time of entry.

The database will contain consolidated information on drug administration, plasma sampling, asparaginase activity data, and drug-associated toxicity. Information

collected in paper CRFs (patient handout sheets, regarding asparaginase doses, schedules, and modifications, if any) will be translated to the database by data entry done in-house. Writing access to the system will be restricted to the authorized staff working within the study team.

Data extraction:

An export from REDCap to datasets suitable for statistical analyses will be performed. The data manager will check that the exported datasets contain all critical information. Critical information per patient is defined as:

- Patient unique ID details
- All necessary information required to calculate and analyze the primary endpoint(s) (including covariates)
- All necessary information required to calculate and analyze key secondary endpoints
- Data will be backed up monthly in .csv format and can be extracted in relevant formats (.csv, .xlsx) as required.

Data validation:

Data validation is the process of testing the validity of data in accordance with the protocol specifications. Edit check programs are written to identify the discrepancies in the entered data, which are embedded in the database, to ensure data validity. These edit check programs are initially tested with dummy data containing discrepancies. Data will be periodically inspected for quality control assessment.

References:

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