


Human Journals

Research Article

May 2022 Vol.:21, Issue:3

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A Preformulation Study of Bendamustine Hydrochloride Injection



IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals



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Submitted: 25 April 2022
Accepted: 30 April 2022
Published: 30 May 2022

Keywords: Bendamustine, anticancer, chronic lymphocytic leukemias (CLL)

ABSTRACT

Bendamustine is a nitrogen mustard [an alkylating agent] that contains a mechlorethamine group and a benzimidazole heterocyclic ring with a butyric acid substituent and used in the treatment of chronic lymphocytic leukemias (CLL) and lymphomas. It belongs to the family of drugs called alkylating agents. It is also being studied for the treatment of sarcoma. Bendamustine was first synthesized in 1963 by Ozegowski and Krebs in East Germany (the former German Democratic Republic). Until 1990 it was available only in East Germany. East German investigators found that it was useful for treating chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma and lung cancer. Bendamustine received its first marketing approval in Germany, which is marketed under the trade name Ribomustin, by Astellas Pharma GmbH's licensee, Mundi Pharma International Corporation Limited, which it is indicated as a single agent or in combination with other anti-cancer agents for indolent NHL, multiple myeloma, and CLL. SymBio Pharmaceuticals Ltd holds exclusive rights to develop and market Bendamustine Hydrochloride in Japan and selected Asia Pacific Rim countries.



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INTRODUCTION:

In March 2008, Cephalon received approval from the United States FDA to market Bendamustine in the US, where it is sold under the trade name Treanda, for treatment of CLL.

As per the literature available, the lengthy exposure of Bendamustine to water during the reconstitution process increases the potential loss of potency and impurity formation due to the hydrolysis of the product by water. In order to control the degradation due to water, an organic solvent called tertiary butyl alcohol is preferred in the formulation.

On Dec 7, 2015, Eagle Pharma received approval from the United States FDA to market Bendamustine in the US, where it is sold under the trade name BENDEKA, for treatment of CLL.

On May 15, 2018, Eagle Pharma received approval from the United States FDA to market Bendamustine in the US, where it is sold under the trade name BELRAPZO, for the treatment of CLL.

The Bendeka and Belrapzo have same composition, same packaging materials and also same fill volume. The only difference is Bendeka is meant for slow infusion whereas the Belrapzo is meant for rapid infusion.

ROUTE OF ADMINISTRATION: Intravenous.

INDICATIONS AND USAGE: Chronic Lymphocytic Leukemia (CLL)

TREANDA® is indicated for the treatment of patients with chronic lymphocytic leukemia. Efficacy relative to first-line therapies other than chlorambucil has not been established.

Non-Hodgkin's Lymphoma (NHL)

TREANDA for Injection is indicated for the treatment of patients with indolent B-cell non-Hodgkin's lymphoma that has progressed during or within six months of treatment with rituximab or a rituximab-containing regimen.

DOSAGE AND ADMINISTRATION DOSING INSTRUCTION FOR CLL:

Recommended Dosage:

The recommended dose is 100 mg/m² administered intravenously over 30 minutes on Days 1 and 2 of a 28-day cycle, up to 6 cycles. Dose Delays, Dose Modifications and Reinitiation of Therapy for CLL:

TREANDA administration should be delayed in the event of Grade 4 hematologic toxicity or clinically significant \geq Grade 2 non-hematologic toxicity. Once non-hematologic toxicity has recovered to \leq Grade 1 and/or the blood counts have improved [Absolute Neutrophil Count (ANC) $\geq 1 \times 10^9/L$, platelets $\geq 75 \times 10^9/L$], TREANDA can be reinitiated at the discretion of the treating physician. In addition, dose reduction may be warranted. Dose modifications for hematologic toxicity:

For Grade 3 or greater toxicity, reduce the dose to 50 mg/m² on Days 1 and 2 of each cycle; if Grade 3 or greater toxicity recurs, reduce the dose to 25 mg/m² on Days 1 and 2 of each cycle. Dose modifications for non-hematologic toxicity:

For clinically significant Grade 3 or greater toxicity, reduce the dose to 50 mg/m² on Days 1 and 2 of each cycle. Dose re-escalation in subsequent cycles may be considered at the discretion of the treating physician. Dosing Instructions for NHL:

Recommended Dosage: The recommended dose is 120 mg/m² administered intravenously over 60 minutes on Days 1 and 2 of a 21-day cycle, up to 8 cycles. Dose Delays, Dose Modifications and Reinitiation of Therapy for NHL:

TREANDA administration should be delayed in the event of Grade 4 hematologic toxicity or clinically significant \geq Grade 2 non-hematologic toxicity. Once non-hematologic toxicity has recovered to \leq Grade 1 and/or the blood counts have improved [Absolute Neutrophil Count (ANC) $\geq 1 \times 10^9/L$, platelets $\geq 75 \times 10^9/L$], TREANDA can be reinitiated at the discretion of the treating physician. In addition, dose reduction may be warranted.

Dose modifications for hematologic toxicity:

For Grade 4 toxicity, reduce the dose to 90 mg/m² on Days 1 and 2 of each cycle; if Grade 4 toxicity recurs, reduce the dose to 60 mg/m² on Days 1 and 2 of each cycle. Dose modifications for non-hematologic toxicity: For Grade 3 or greater toxicity, reduce the dose to 90 mg/m² on Days 1 and 2 of each cycle; if Grade 3 or greater toxicity recurs, reduce the dose to 60 mg/m² on Days 1 and 2 of each cycle.

RECONSTITUTION/PREPARATION FOR INTRAVENOUS ADMINISTRATION

- Aseptically reconstitutes each vial as follows:
- 25 mg vial: Add 5 mL of only Sterile Water for Injection, USP.
- 100 mg vial: Add 20 mL of only Sterile Water for Injection, USP.

Shake well to yield a clear, colorless to a pale-yellow solution with a Bendamustine HCl concentration of 5 mg/mL. The lyophilized powder should completely dissolve in 5 minutes. If particulate matter is observed, the reconstituted product should not be used. Aseptically withdraw the volume needed for the required dose (based on 5 mg/mL concentration) and immediately transfer to a 500 mL infusion bag of 0.9% Sodium Chloride Injection, USP (normal saline). As an alternative to 0.9% Sodium Chloride Injection, USP (normal saline), a 500 mL infusion bag of 2.5% Dextrose/0.45% Sodium Chloride Injection, USP, may be considered. The resulting final concentration of bendamustine Hydrochloride in the infusion bag should be within 0.2 – 0.6 mg/mL. The reconstituted solution must be transferred to the infusion bag within 30 minutes of reconstitution. After transferring, thoroughly mix the contents of the infusion bag. The admixture should be clear and colorless to a slightly yellow solution. Use Sterile Water for Injection, USP, for reconstitution and then either 0.9% Sodium Chloride Injection, USP, or 2.5% Dextrose/0.45% Sodium Chloride Injection, USP, for dilution, as outlined above. No other diluents are compatible.

MECHANISM OF ACTION:

Bendamustine is a bifunctional mechlorethamine derivative containing a purine-like benzimidazole ring. Mechlorethamine and its derivatives form electrophilic alkyl groups. These

groups form covalent bonds with electron-rich nucleophilic moieties, resulting in interstrand DNA crosslinks. The bifunctional covalent linkage can lead to cell death via several pathways. Bendamustine is active against both quiescent and dividing cells. The exact mechanism of action of bendamustine remains unknown.

PHARMACOKINETICS:

Absorption:

Following a single IV dose of bendamustine hydrochloride C_{max} typically occurred at the end of infusion. The dose proportionality of Bendamustine has not been studied.

Distribution:

In vitro, the binding of bendamustine to human serum plasma proteins ranged from 94-96% was concentration independent from 1-50 µg/mL. Data suggest that bendamustine is not likely to displace or to be displaced by highly protein-bound drugs. The blood to plasma concentration ratios in human blood ranged from 0.84 to 0.86 over a concentration range of 10 to 100 µg/mL indicating that bendamustine distributes freely in human red blood cells. In humans, the mean steady-state volume of distribution (V_{ss}) was approximately 25 L.

Metabolism:

In vitro data indicate that bendamustine is primarily metabolized via hydrolysis to metabolites with low cytotoxic activity. In vitro, studies indicate that two active minor metabolites, M3 and M4, are primarily formed via CYP1A2. However, concentrations of these metabolites in plasma are 1/10 and 1/100 that of the parent compound, respectively, suggesting that the cytotoxic activity is primarily due to bendamustine. In vitro studies using human liver microsomes indicate that bendamustine does not inhibit CYP1A2, 2C9/10, 2D6, 2E1, or 3A4/5. Bendamustine did not induce the metabolism of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, or CYP3A4/5 enzymes in primary cultures of human hepatocytes.

Elimination:

No mass balance study has been undertaken in humans. Preclinical radiolabeled bendamustine studies showed that approximately 90% of drug administered was recovered in excreta primarily

in the feces. Bendamustine clearance in humans is approximately 700 mL/minute. After a single dose of 120 mg/m² bendamustine IV over 1-hour the intermediate t_{1/2} of the parent compound is approximately 40 minutes. The mean apparent terminal elimination t_{1/2} of M3 and M4 is approximately 3 hours and 30 minutes respectively. Little or no accumulation in plasma is expected for bendamustine administered on Days 1 and 2 of a 28-day cycle.

STORAGE CONDITION: US Geography:

TREANDA may be stored up to 25°C (77°F) with excursions permitted up to 30°C (86°F) (see USP Controlled Room Temperature). Retain in original package until time of use to protect from light.

Rational for Drug Candidate Selection

Bendamustine Hydrochloride for Injection is an alkylating drug indicated for the treatment of patients with:

- Chronic lymphocytic leukemia (CLL). And in
- Indolent B-cell non-Hodgkin's lymphoma (NHL) that has progressed during or within six months of treatment with rituximab or a rituximab-containing regimen. Which is a first kind of drug proposed for the usage as per above

The drug candidate has shown challenges to develop a stable and product having lower levels of impurity profiles.

Since the drug candidate is easily liable for hydrolytic degradation, it becomes an opportunity to use tertiary Butanol as a co-solvent to use in the Bendamustine HCl formulation.

Before initiating the developmental work with the drug candidate, it is important to determine certain fundamental physical and chemical properties of the drug molecule and other derived properties of the drug. The early-stage developmental activities are very important for successful formulation work. This information will dictate many of the subsequent events and possible approaches in the development of the formulation.

Description

The physical appearance of Bendamustine Hydrochloride was observed and reported in the results section.

Identification by IR spectroscopy

Fourier Transform Infra-Red Spectrum (FTIR)

Transferred about a small quantity of KBr into agate mortar, triturate evenly, and performed as the blank disc. Triturated about 1% Bendamustine hydrochloride into powdered KBr evenly to again a sample disc scan blank flake and sample flake and record the chromatogram.

The FT-IR spectral measurements were taken at ambient temperature using an FT-infrared spectrophotometer Shimadzu, 8400. About 2 mg of the pure drug was selected separately. The pure drug was dispersed in KBr powder and the pellets were made by applying pressure. FT-IR spectra were obtained by powder diffuse reflectance on an FT-IR spectrophotometer. Identification is done by Shimadzu, 8400. Scanning range was 400 cm^{-1} to 4000 cm^{-1} .

Solubility determination

A small quantity of Bendamustine Hydrochloride was dissolved in each of the following solvents:

Water, N-methyl pyrrolidone [NMP], Dimethylformamide [DMF], Dimethylacetamide [DMA], Propylene glycol, Polyethylene glycol 400 and Dimethylsulfoxide [DMSO]. The aqueous solubility of Bendamustine hydrochloride was studied in different pH buffers and reported in the results section.

Table No. 1: Showing the USP definition of solubility

USP standards	
Soluble	10 to 30 parts of solvent
Sparingly soluble	30 to 100 parts solvent
Slightly soluble	100 to 1000 parts of
Insoluble	10,000 parts of solvents

Melting point:

A small quantity of Bendamustine Hydrochloride was filled into a capillary tube with one end sealed and kept into the socket of the apparatus. Then the sample was observed for melting point determination. The procedure was repeated 3 times and an average of the same was reported. However, the melting point was also determined by the DSC thermogram method.

Melting Point: The melting point determination was also done by using the DSC method. The result and the DSC chromatogram are reported in the results section.

Water content:

Karl Fischer titration is a classic titration method in analytical chemistry that uses coulometric or volumetric titration to determine trace amounts of water in a sample.

Karl Fischer titration was used as an analytical method for quantifying water content in the API and drug and methanol were used as a solvent.

$$\text{Moisture content} = \frac{\text{Volume consumed} \times \text{KF factor}}{\text{Weight taken}}$$

The water content was checked by auto Karl Fischer Titration and the results were reported.

pH (0.5% solution in water): pH of the 1% solution in water was determined using pH meter and the result is reported.

Hygroscopicity: The drug substance is light hygroscopic and environmental conditions such as temperature and humidity induce polymorphic conversions. A hygroscopic study was performed to ascertain the relative stability of polymorphs with respect to standard form by applying the relative stability of accelerated environmental conditions (i.e 75%RH at 40°C). Samples were accurately weighed and stored at 75% relative humidity (RH) at 40°C. The weight of samples was measured after 7, 14 and 28 days.

Table No. 2: PREFORMULATION STUDY RESULTS

S.No.	Test parameter	Results																		
1	Description	White to off white crystalline powder of Bendamustine hydrochloride was observed																		
2	Identification by IR	The sample spectrum exhibited maxima only at the same wave length as that of the standard spectrum. The IR spectrum of Bendamustine hydrochloride																		
3	Melting point by capillary	Between 160° to 163°C.																		
4	Melting point by DSC	About 157°C (DSC data attached)																		
5	Solubility	<table border="1"> <thead> <tr> <th>Solvent</th> <th>mg/mL [assay]</th> </tr> </thead> <tbody> <tr> <td>Water</td> <td>15.00</td> </tr> <tr> <td>N-methyl pyrrolidone [NMP]</td> <td>104.0</td> </tr> <tr> <td>Dimethylformamide [DMF]</td> <td>70.00</td> </tr> <tr> <td>Dimethylacetamide [DMA]</td> <td>55.00</td> </tr> <tr> <td>Propylene glycol</td> <td>45.00</td> </tr> <tr> <td>Polyethylene glycol 400</td> <td>Nil</td> </tr> <tr> <td>Dimethyl sulfoxide [DMSO]</td> <td>300.00</td> </tr> <tr> <td>Methanol</td> <td>1000.00</td> </tr> </tbody> </table>	Solvent	mg/mL [assay]	Water	15.00	N-methyl pyrrolidone [NMP]	104.0	Dimethylformamide [DMF]	70.00	Dimethylacetamide [DMA]	55.00	Propylene glycol	45.00	Polyethylene glycol 400	Nil	Dimethyl sulfoxide [DMSO]	300.00	Methanol	1000.00
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6.0	5 mg/mL																			
8.0	5 mg/mL																			
6	Water content	4.37%																		
7	pH	3.12																		
8	Hygroscopic study	The material found not much hygroscopic																		
9	XRD study	The result conformed to the standard used in the test and conforming to the polymorph																		
10	FDM microscopic results	Collapse temperature is -26.2°C Freezing initiation is -31.4°C																		

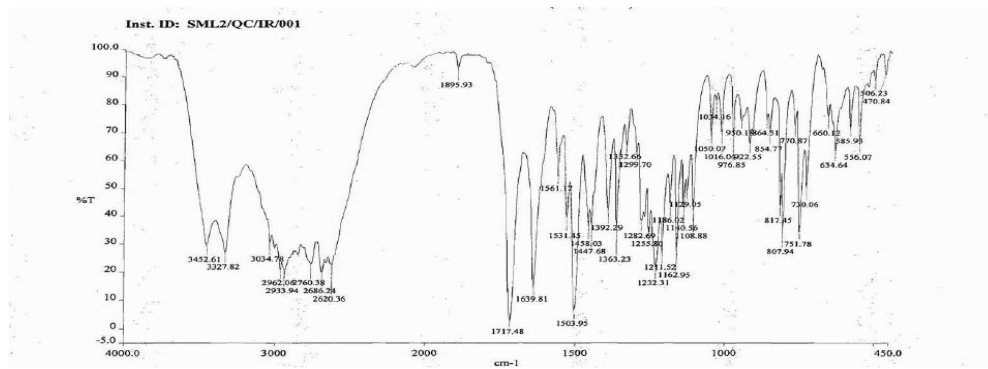


Fig No. 1: Identification by IR

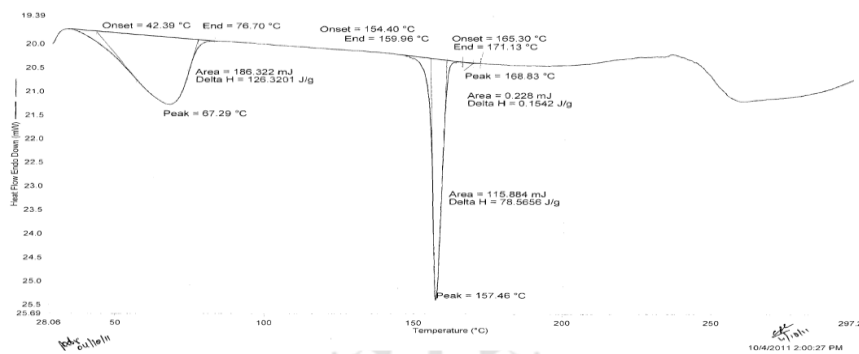


Fig No. 2: Melting point by DSC chromatogram

Table No.: 3 Hygroscopic Study Results:

API	Weight (mg)				Weight Change (%)			
	0 Day	7 th Day	21 st Day	28 th Day	0 Day	7 th Day	21 st Day	28 th Day
Bendamustine Hydrochloride	500.00	502.80	502.4	503.1	Nil	0.56%	0.48%	0.62%

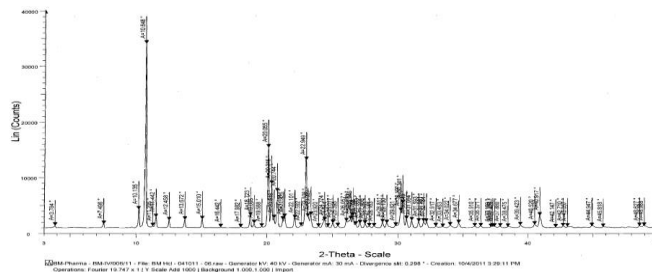


Fig No. 3: XRD Chromatogram

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