

# Cytomegalovirus Occurrence and Time-to-onset Analysis Under Bendamustine With Anti-CD20 Antibodies Using the JADER Database

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**Abstract.** *Background/Aim:* Patients with malignant lymphoma, in a latent state of weakened immune function, are at risk of chemotherapy-induced immunosuppression and cytomegalovirus (CMV) infection. Concomitant therapy with bendamustine and rituximab or obinutuzumab intensifies immunosuppression, potentially affecting CMV onset. This study aimed to assess CMV onset differences between bendamustine monotherapy and combination therapy with rituximab or obinutuzumab using the Japanese Adverse Drug Event Report database (JADER). *Patients and Methods:* A JADER analysis dataset (April 2004 to September 2022) defined CMV infection using 31 preferred term (PT) words from MedDRA 25.1J HLT “Cytomegalovirus infection (10011827)”. Reporting odds ratios (ROR) calculated CMV infection signals for bendamustine monotherapy, rituximab, obinutuzumab, bendamustine+rituximab (BR), and bendamustine+obinutuzumab (GB). ROR confidence intervals exceeding 1 indicated a CMV signal. Days of CMV infection were calculated based on adverse event onset and administration start. *Results:* CMV signals were confirmed for monotherapy and combination therapies. CMV infection durations (median, interquartile range) were 41.0 days (23.5-69.5) for bendamustine monotherapy, 63.5 days (35.2-95.0) for

BR, and 61.0 days (33.0-102.5) for GB, with cases exceeding 200 days. *Conclusion:* JADER analysis detected significant CMV signals for rituximab, obinutuzumab, and bendamustine. Caution may be warranted 7-9 months post-bendamustine administration, necessitating further investigation, including cell-mediated immunity suppression assessment.

In malignant lymphoma, a tumor of normal lymphocytes, patients experience immunosuppression from the primary disease. Systemic chemotherapy with anticancer drugs, including Bendamustine (Benda), exacerbates immune function impairment. Benda, with nitrogen mustard chemical structures and purine analog-like structures, induces potent immunosuppression, causing prolonged T-cell lymphopenia (1, 2). Furthermore, Benda monotherapy poses infection risks due to T-cell lymphopenia. Combining it with anti-CD20 monoclonal antibodies, such as rituximab (RIT) and obinutuzumab (Obi), requires caution due to B cell depression and increased infection susceptibility.

Human cytomegalovirus (CMV), a DNA virus of the herpesvirus family, poses a fatal threat to certain populations, particularly immunosuppressed and hematological patients. Acute CMV infection significantly impacts morbidity and mortality in these patients (3), with reports linking it to malignant lymphoma (4, 5), sometimes leading to severe outcomes. Normal T lymphocyte function is crucial for controlling CMV reinfection. The use of purine analogs, potent T lymphocyte suppressors that are effective in treating chronic lymphoproliferative disorders (6), is pertinent to CMV reactivation development.

Recent years have witnessed advancements in pharmacovigilance approaches for detecting drug-associated adverse event (AE) signals using large databases, such as the Japanese Adverse Drug Event Report (JADER), based on spontaneous AE reports (7, 8). Evaluation of drug-associated AE signals involves disproportionality analysis, including

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**Key Words:** Bendamustine, anti-CD20 monoclonal antibodies, cytomegalovirus infection, Japanese Adverse Drug Event Report database.



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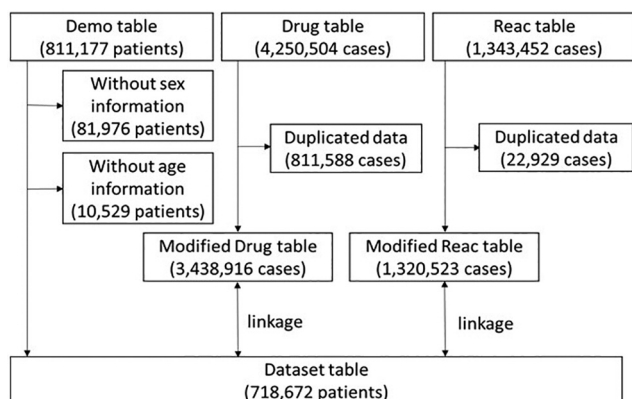


Figure 1. Flow diagram of the study.

calculating reporting odds ratios (RORs) and information components (ICs) in pharmacovigilance activity (7). JADER, a nationwide open-access database, compiles spontaneous AE reports from the Pharmaceuticals and Medical Devices Agency (PMDA), a pharmaceutical regulatory authority in Japan.

CMV infections have been reported in lymphoma patients treated with Benda and anti-CD20 antibody in a real-world study (9). However, it remains unclear whether CMV infection signaling occurs in patients treated with Benda monotherapy or Benda in combination with anti-CD20 monoclonal antibodies. In addition, there are no reports on the clinically important time from Benda administration to the onset of CMV using JADER.

This study aimed to investigate CMV infection signals during Benda monotherapy or Benda in combination with anti-CD20 monoclonal antibodies, using the JADER database, as well as the time to onset of CMV infections in patients treated with Benda monotherapy or Benda combined with anti-CD20 monoclonal antibodies.

## Patients and Methods

**Data source.** Data from the JADER database, spanning April 2004 to September 2022, were obtained from the PMDA website (1). The JADER dataset included four tables: demographic information (“demo”), drug information (“drug”), and adverse event information (“reac”). It comprised 811,177 patients, 4,250,504 cases, and 1,343,452 adverse events. The “demo” table contained patient sex and age data. Patients with blank or unknown sex or age data in the “demo” table, and notifications with duplicated data in the “drug”, “reac”, and “hist” tables, were excluded. The “demo” table was linked to the “drug”, “reac”, and “hist” tables using patients’ identification numbers. After data cleaning, 718,672 patients were included in this study (Figure 1).

**Definition of cytomegalovirus infections.** CMV infections were extracted from the “reac” table based on preferred terms (PTs) in the Medical Dictionary for Regulatory Activities (MedDRA, 25.0J). Thirty-one PTs, categorized under the high-level term (HLT)

Table I. Definition of cytomegalovirus infections.

HLT code	HLT name
10011827	Cytomegaloviral infections
PT code	PT name
10010430	Congenital cytomegalovirus infection
10011830	Cytomegalovirus hepatitis
10011831	Cytomegalovirus infection
10011834	Cytomegalovirus mononucleosis
10014586	Encephalitis cytomegalovirus
10035676	Pneumonia cytomegaloviral
10048843	Cytomegalovirus chorioretinitis
10048983	Cytomegalovirus colitis
10049014	Cytomegalovirus duodenitis
10049015	Cytomegalovirus enterocolitis
10049016	Cytomegalovirus gastritis
10049018	Cytomegalovirus esophagitis
10049074	Cytomegalovirus enteritis
10049075	Disseminated cytomegaloviral infection
10049566	Cytomegalovirus pancreatitis
10051349	Cytomegalovirus gastroenteritis
10051350	Cytomegalovirus urinary tract infection
10052817	Cytomegalovirus gastrointestinal infection
10056261	Cytomegalovirus myocarditis
10056262	Cytomegalovirus syndrome
10056721	Cytomegalovirus pericarditis
10058666	Cytomegalovirus infection reactivation
10058854	Cytomegalovirus viraemia
10065036	Cytomegalovirus mucocutaneous ulcer
10065621	Cytomegalovirus myelomeningoradiculitis
10075619	Cytomegalovirus gastrointestinal ulcer
10079095	Cytomegalovirus nephritis

HLT: High level term; PT: preferred term.

“Cytomegaloviral infections” (HLT code 10011827), were identified as CMV infections (Table I).

**Signal detection.** This study utilized ROR and IC for CMV signal detection, as previously reported (10-13). ROR serves as an AE signal index, representing the odds ratio of reporting a specific AE versus all other AEs related to the target drugs, compared to odds for all other drugs. However, ROR results may be unreliable with a small sample size. IC, an adverse drug reactions (ADR) signal index from Bayesian Confidence Propagation Neural Network analysis, can detect AE signals even with a small sample size (10, 14). RORs, ICs, and their 95% confidence intervals (CIs) were calculated using a two-by-two contingency table (Table II) and the provided equations (15).

ROR equations:

$$ROR = \frac{N_{11}/N_{01}}{N_{10}/N_{00}} = \frac{N_{11}N_{00}}{N_{10}N_{01}}$$

$$ROR(95\% CI) = e^{\ln(ROR) \pm 1.96 \sqrt{\frac{1}{N_{11}} + \frac{1}{N_{10}} + \frac{1}{N_{01}} + \frac{1}{N_{00}}}}$$

IC Equations:

$$E(IC_{11}) = \log_2 \frac{(N_{11} + \gamma_{11})(N_{++} + \alpha)(N_{++} + \beta)}{(N_{++} + \gamma)(N_{1+} + \alpha_1)(N_{+1} + \beta_1)}$$

$$V(IC_{11}) = \left(\frac{1}{\ln 2}\right)^2 \left[ \frac{N_{++} - N_{11} + \gamma - \gamma_{11}}{(N_{11} + \gamma_{11})(1 + N_{++} + \gamma)} + \frac{N_{++} - N_{1+} + \alpha - \alpha_1}{(N_{1+} + \alpha_1)(1 + N_{++} + \alpha)} + \frac{N_{++} - N_{+1} + \beta - \beta_1}{(N_{+1} + \beta_1)(1 + N_{++} + \beta)} \right]$$

Table II. Two-by-two contingency table.

	Target AEs	Other AEs	Total
Target drugs	$N_{11}$	$N_{10}$	$N_{1+}$
Other drugs	$N_{01}$	$N_{00}$	$N_{0+}$
Total	$N_{+1}$	$N_{+0}$	$N_{++}$

AEs: Adverse events; N: number of cases.

$$\gamma = \gamma_{11} \frac{(N_{++} + \alpha)(N_{++} + \beta)}{(N_{1+} + \alpha_1)(N_{+1} + \beta_1)}, \gamma_{11} = 1, \alpha_1 = \beta_1 = 1, \alpha = \beta = 2$$

$$IC(95\% CI) = E(IC_{11}) \pm 2\sqrt{V(IC_{11})}$$

The calculations were performed using Microsoft Excel (Microsoft Japan Co., Ltd., Tokyo, Japan). IOH signals were positive if the 95%CI lower limit of ROR exceeded 1 and IC exceeded 0.

*Time-to-onset analysis.* Time-to-onset analysis utilized periods from the initial administration of RIT, Obi, and Benda in the “drug” table to the date of the first CMV infection occurrence in the “reac” table. Patients with missing or inaccurate data on initial administration and CMV infection dates were excluded. Histograms depicted time and frequency, and median period and interquartile range (IQR) were calculated and presented using the R Statistical Package (version 3.5.2; The R Foundation for Statistical Computing, Vienna, Austria) (16).

**Results**

*CMV infections signal under anti-CD20 monoclonal antibodies and Benda therapy.* The RORs and ICs of CMV infections for anti-CD20 monoclonal antibodies and Benda therapy are presented in Table III. Drugs encompassed all reports of RIT, Obi, Benda, and combined therapies (BR: RIT+Benda, GB: Obi+Benda, and Benda alone). Significant AE expression signals were detected in RIT, Obi, and Benda. BR, GB, and Benda monotherapy also showed significant AE expression signals.

*Time-to-onset analysis.* In the time-to-onset analysis, the median period (IQR) for CMV infection onset in patients with BR, GB, and Benda monotherapy were as follows: BR therapy [63.5 (35.2-95.0) days], GB therapy [61.0 (33.0-102.5) days], and Benda monotherapy [41.0 (23.5-69.5) days].

*BR therapy.* Figure 2A illustrates CMV infection onset time and frequency following BR therapy. Seventy cases were reported, excluding 25 with unknown details. The most frequent onset time was 80-89 days, with 10 reports, and some cases occurred on 210-229 days.

*GB therapy.* Figure 2B shows CMV infection onset time and frequency following GB therapy. Nineteen cases were reported, excluding 14 with unknown details. Similar to BR

Table III. The reporting odds ratios (RORs) and information components (ICs) of cytomegalovirus (CMV) infections following anti-CD20 monoclonal antibodies and bendamustine therapy.

Drugs	CMV infection cases	ROR [95%CI]	IC [95%CI]
Rituximab	376	16.26 [14.53-18.19]	3.68 [3.52-3.85]
Obinutuzumab	43	14.46 [10.62-19.69]	3.40 [2.95-3.85]
Bendamustine	174	18.48 [15.77-21.65]	3.90 [3.67-4.13]
RIT+Benda (BR)	93	17.93 [13.77-21.07]	3.75 [3.44-4.06]
Obi+Benda (GB)	33	14.62 [10.28-20.78]	3.32 [2.81-3.83]
Benda monotherapy	47	22.23 [16.48-29.97]	3.85 [3.42-4.29]

RIT: Rituximab; Obi: obinutuzumab.

therapy, in addition to 80-89 days, the frequency was notable in the early 20-29 days after Benda administration. There was also a report of 220-229 days after Benda administration.

*Benda monotherapy.* Figure 2C displays the histogram of CMV infection onset time and frequency following Benda administration in monotherapy. Forty-three cases were reported, excluding 4 with unknown details. The most common onset occurred 40-49 days after Benda administration, with 10 cases. Additionally, six reports were within days 0-9, and a report specifically mentioned Benda alone. One report indicated an onset after 234 days of treatment.

**Discussion**

Signals of CMV infection were evident in RIT, Obi, Benda monotherapy, and combinations with RIT and Obi. Alongside lymphoma’s pathological condition, the combined use of anti-CD20 antibodies, specifically suppressing lymphocytes, and Benda, reducing cell-mediated immunity, posed infection risks.

CMV infection is crucial in immunosuppressed and hematological patients. Acute infection causes substantial morbidity and mortality in these patients. Thus, timing information about CMV infection onset is valuable for prevention in hematological patients.

From the time-to-onset analysis, reports of CMV development over 200 days after were observed in patients treated with BR, GB, and Benda monotherapy. The decrease in cell-mediated immunity caused by Benda implies a reduction in CD4-positive cells. CD4-positive cell count dropped below 200/μl after one course of Benda administration, persisting for 7 to 9 months post-treatment (1). This study also reports CMV infection development 7-8 months after Benda administration, aligning with the period of CD4-positive cell count suppression.

Compared to RIT, a type I antibody, Obi is a type II antibody (17). Modifications in the elbow hinge region

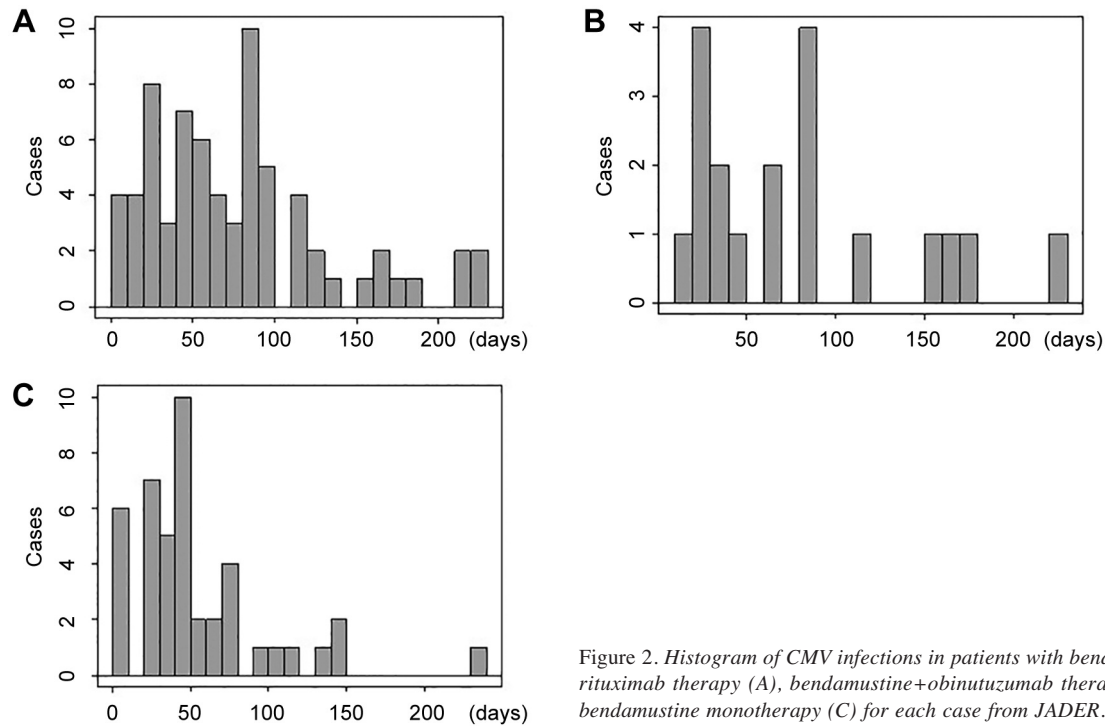


Figure 2. Histogram of CMV infections in patients with bendamustine+rituximab therapy (A), bendamustine+obinutuzumab therapy (B) and bendamustine monotherapy (C) for each case from JADER.

confer higher direct cell death-inducing activity (18). Additionally, Obi exhibits improved binding affinity with Fcγ receptor III, enhancing antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) (19, 20) compared to RIT. Obi is considered to have higher anti-CD20 activity, as supported by the GALLIUM trial results, demonstrating a statistically significant extension in PFS compared to rituximab-based chemotherapy (R-chemo) in initial-onset FL (20). The GADOLIN trial revealed that GB therapy significantly extended PFS compared to Benda monotherapy for rituximab-resistant indolent non-Hodgkin lymphoma (iNHL) (21). In comparison to R-chemo, obinutuzumab-based chemotherapy is significantly effective but exhibits a higher occurrence of infusion reactions and infection, necessitating careful side-effect management (20, 21). In the GALLIUM trial, infection rates were 77.3% in the Obi group and 70.0% in the RIT group. However, while the Obi group had no cases of viral infection, the RIT group reported three cases (0.5%). The GADOLIN trial noted 11 cases (6%) of overall infection Grade 3 or higher for GB therapy and 16 cases (9%) for Benda monotherapy. Cytomegalovirus chorioretinitis occurred once for both GB and Benda monotherapy. Our time-to-onset analysis revealed that the median onset time tended to be lower for both BR and GB therapy compared to Benda monotherapy.

The efficacy of Obi suggests stronger antilymphocyte action-based immunosuppression and its prolongation

compared to that of RIT, although clear data are lacking. Our reports indicate CMV occurrences seven months after B administration in BR, GB, and B monotherapy. Caution is necessary, especially for elderly patients with a high immunosuppression risk, requiring regular CD4-positive cell count measurements and implementation of infection prevention measures.

*Study limitations.* First, JADER, being a spontaneous reporting system, is passive and prone to biases, including under-reporting, over-reporting, and comorbidity confounding. Second, JADER's characteristics make it challenging to assess the number of treatment courses and chemotherapy drug dosage. Finally, the time-to-onset analysis had a small number of CMV infection cases, requiring caution in result interpretation.

## Conclusion

In the JADER report on CMV infection, significant adverse event signals were found for RIT, Obi, and Benda. Clinical caution is needed for 7-9 months post-Benda administration. Further investigation, including assessing the state of cell-mediated immunity suppression, is necessary.

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## Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

## Authors' Contributions

Kaori Ito and Takenao Koseki designed the study. Kaori Ito and Takenao Koseki conducted data analysis. Kaori Ito and Takenao Koseki drafted the manuscript. Misaki Morisaku, Shigeki Yamada and Nobuki Hayakawa reviewed the manuscript. All the Authors approved the final manuscript.

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