

Complete Response (CR) in a Previously-progressing Chronic Lymphocytic Leukemia (CLL) Patient Treated With Methionine Restriction in Combination With First-line Chemotherapy

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Abstract. *Background/Aim:* Chronic lymphocytic leukemia (CLL) is currently incurable. CLL is characterized by disordered DNA methylation. The aim of the present study was to target methylation with methionine restriction in a patient with progressive CLL. *Case Report:* Methionine restriction for the patient was achieved with a low-methionine vegan diet and oral recombinant methioninase (o-rMETase). The patient also received rituximab, once per week for four weeks, and acalabrutinib 100 mg, twice daily (bid) continuously. The patient's white blood cell count decreased by 95% from peak levels and extensive lymphadenopathy disappeared during combination treatment with o-rMETase, rituximab, and acalabrutinib. *Conclusion:* The combination of methionine restriction and first-line chemotherapy resulted in an apparent complete response (CR) in a CLL patient, a rare event. The duration of the CR will be monitored, and additional CLL patients will be treated similarly in the future.

Chronic lymphocytic leukemia (CLL) is the result of overproduction of B lymphocytes which accumulate both in the blood circulation and in lymph nodes (1). CLL is characterized by a decrease in global DNA methylation compared with normal B-lymphocytes, along with increased DNA methylation of CpG islands (2) that may decrease the expression of genes that are necessary for drug sensitivity (3). Global DNA hypomethylation in cancer was discovered by one of us (RMH) in 1982 (4).

First-line therapy for CLL includes inhibitors of Bruton's tyrosine kinase (BTK), such as ibrutinib and acalabrutinib, as well as rituximab, fludarabine and cyclophosphamide (5-8). However, CLL remains incurable, despite patient responses, which generally are not durable (9).

Given that methylation is aberrant in CLL, we treated a CLL patient who was rapidly progressing with methionine restriction (MR) effected by oral recombinant methioninase (o-rMETase) and a low-methionine diet combined with first-line chemotherapy, resulting in an apparent complete response.

Case Report

The patient is an 85-year-old female that was diagnosed with CLL in 2019 while being treated for an abdominal sarcoma. The patient began MR, including an MR diet and was also treated with o-rMETase (10-16), which was purified from recombinant *E. coli* containing the *Pseudomonas putida* methioninase gene, as previously described (17). The patient received o-rMETase after each meal (250 units/1 ml). The patient was also treated with rituximab q week $\times 4$ and acalabrutinib 100 mg twice daily (bid) continuously.

The patient started o-rMETase and the MR diet in September 2022. At this time, her white blood cell (WBC) count was $108 \times 10^3/\mu\text{l}$. The WBC continued to rise, reaching

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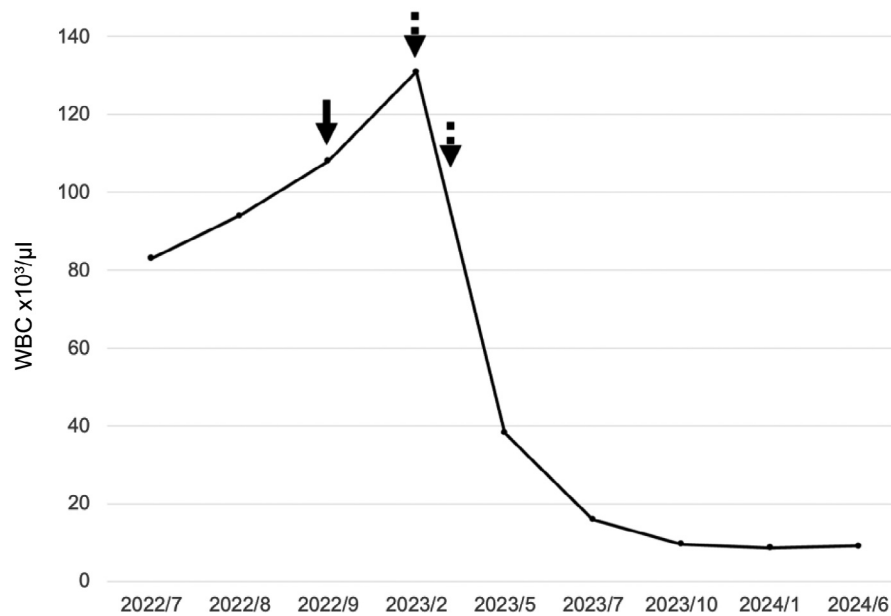


Figure 1. Time course of changes in white blood cell (WBC) levels ($\times 10^3/\mu\text{l}$) in a patient with chronic lymphocytic leukemia (CLL) treated with acalabrutinib, rituximab, oral methioninase (o-rMETase), and a low methionine diet. Solid arrow indicates the start of rMETase and low-methionine diet. Dashed arrows indicate the start of rituximab (2023/2) and acalabrutinib (2023/3).

$195 \times 10^3/\mu\text{l}$ by February 2023, at which point rituximab was initiated for one month. In March 2023, acalabrutinib was initiated, leading to a steep decline in the WBC count, which reached $8.1 \times 10^3/\mu\text{l}$ in December 2023, which is within the normal range. WBC counts were $8.6 \times 10^3/\mu\text{l}$ in January 2024 and $9.1 \times 10^3/\mu\text{l}$ in June 2024 (Figure 1).

In January 2023, the patient had enlarged mediastinal and right-axillary-lymph-node adenopathy. For example, a right axillary lymph node was 18 mm in the short axis and a paratracheal lymph node was 9 mm in the short axis, observed using chest/abdominal/pelvis computed tomography (CT) with intravenous injection of a contrast agent.

By November 2023, no evidence of lymphadenopathy or disease progression was observed in the chest, abdominal or pelvis on CT scans. An apparent complete response (CR) was assumed based on the WBC count and CT of the lymph nodes. The CR appears durable as the patient continues to have a low WBC count as of September 2024.

Discussion

CLL is indolent in some patients and aggressive in others. It is considered incurable (21). CLL is characterized by disordered DNA methylation, which can cause clonal heterogeneity, including drug resistance and more aggressive progression (2).

In the present study, at the start of MR, with o-rMETase and the MR diet, the patient continued to progress. However, when acalabrutinib was added to MR, the patient's WBC count

rapidly declined to normal, and lymphadenopathy disappeared in an apparent CR. The present study suggests that MR in combination with first-line chemotherapy was effective.

Very recent studies suggested that inhibiting the anti-apoptotic protein BCL-2 in CLL via venetoclax in combination with the BTK inhibitor ibrutinib increases survival in CLL compared to the combination of fludarabine, cyclophosphamide and rituximab (FCR) (19). MR could be added to this regimen as well. A new BTK inhibitor for CLL, pirobrutinib, has also shown promising results (20).

MR is synergistic with all types of chemotherapy in preclinical mouse models of cancer including patient-derived orthotopic xenograft (PDOX) models (12-46). MR combined with chemotherapy may be synergistic in the clinic as suggested by the present and previous reports (12-16, 22, 46).

MR is effective as it targets the fundamental and universal hallmark of cancer, methionine addiction, termed the Hoffman effect (21, 47-68).

Conflicts of Interest

The Authors declare no competing interests in relation to this study.

Authors' Contributions

SM was a major contributor to writing the manuscript and RMH revised the paper. QH produced o-rMETase, KM, BMK, MS, MB, NY, KH, HK, SM, KI, TH, HT and SD critically read and approved the final manuscript.

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