Severe hemolysis and transfusion reactions after treatment with BGB-3111 and PD-1 antibody for Waldenström macroglobulinemia

We describe two patients with relapsed Waldenström Macroglobulinemia (WM) treated with a novel programmed cell death protein 1 (PD-1) antibody (BGB-A317) in combination with BGB-3111, a Bruton's tyrosine kinase (BTK) inhibitor, as part of a Phase 1b dose-escalation study (*clinicaltrials.gov identifier 02795182*). Both patients developed severe direct antiglobulin test (DAT) negative hemolysis and reticulocytopenia associated with transfusions. BTK inhibitors, including BGB-3111, are known to be safe and highly active in WM.[1,2] Immune checkpoint inhibitors such as antibodies against PD-1 have been increasingly used in the management of solid organ and lymphoid malignancies, and are associated with a spectrum of immune-related adverse events, including hematological effects.[3] PD-1 and its ligands PD-L1 and PD-L2 are expressed on malignant B cells in WM, providing a rationale for the use of PD-1 inhibitors in this condition.[4-6]

Patient 1 is a 62-year-old female diagnosed with WM in 2011. She was treated at the time of diagnosis with rituximab, cyclophosphamide and dexamethasone, attaining a complete response (CR). At first relapse 21 months later, she received rituximab and bendamustine with another CR. In late 2016, she developed anemia (hemoglobin 90 g/L), thrombocytopenia (121x10^9/L) and a rising IgM (18.1 g/L), without lymphadenopathy or splenomegaly. A bone marrow aspirate and trephine confirmed relapsed WM.

The patient was enrolled on a clinical trial and started BGB-3111, followed one and four weeks later by BGB-A317 infusions. Four weeks after the first dose of the PD-1 antibody, she developed hemoglobinuria, flank pain and fevers during a red cell transfusion. Biochemical markers of hemolysis were abnormal (bilirubin 80 µmol/L, lactate dehydrogenase [LDH] 598 U/L, haptoglobin <0.2 g/L), but the DAT was negative and blood film unremarkable. No allo- or auto-antibodies against red cell antigens were identified.

No further doses of BGB-A317 were given. The patient required 26 further red cell transfusions for symptomatic anemia, with ongoing hemolysis associated with a mild acute kidney injury and intermittent symptomatic transfusion reactions. Of note, she was profoundly reticulocytopenic throughout this time despite a relatively normal reticulocyte count prior to enrolment. Parvovirus serology was negative and a repeat bone marrow biopsy showed markedly reduced erythropoiesis and ongoing involvement by WM. Flow cytometric analysis of the lymphocyte compartment showed that the B cells (52% of lymphocytes) consisted entirely of clonal B cells with lambda light chain restriction. The T cells had a CD4:CD8 ratio of 1:1 and there were very few NK cells.

Prednisolone at 1mg/kg was subsequently initiated with a rapid and marked improvement in hemoglobin, reticulocytes and hemolytic markers. The corticosteroids were weaned and she remains 8 months transfusion independent. At the time of analysis her WM was responding to single agent BTK inhibitor with IgM of 7.1 g/L (partial response).

Patient 2 is a 67-year-old female with insulin-dependent diabetes mellitus diagnosed with WM in 2006. Initial treatment was rituximab, fludarabine, and cyclophosphamide followed by maintenance rituximab,

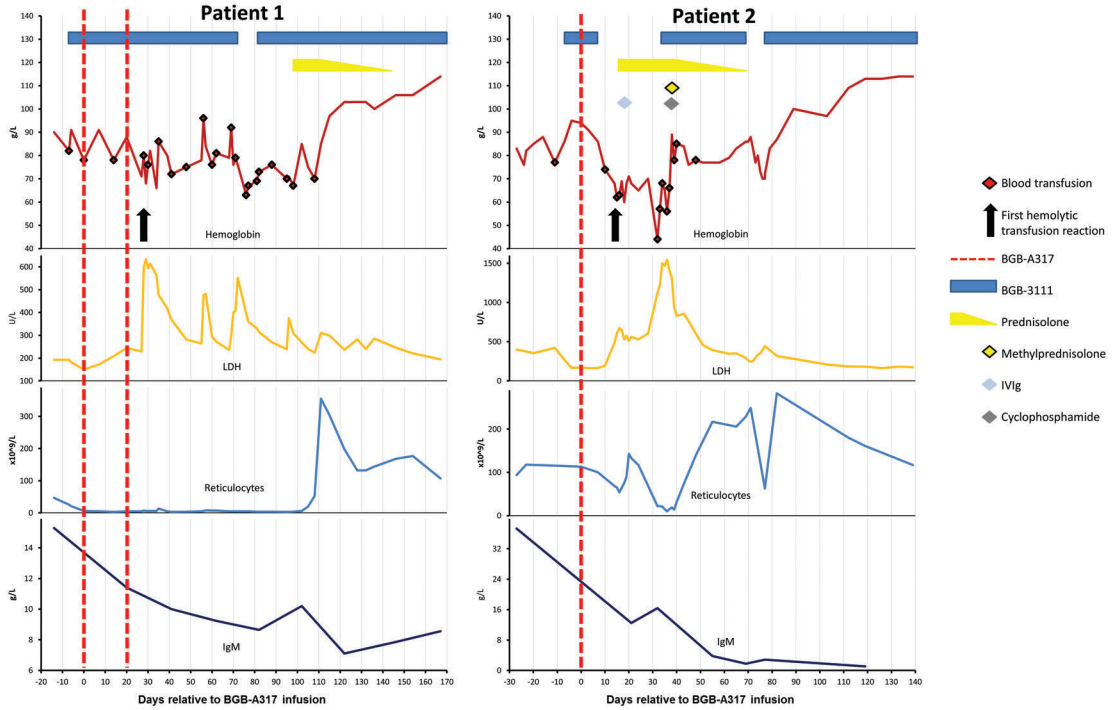

Figure 1. Chart of hemoglobin, lactate dehydrogenase (LDH), reticulocyte count and IgM relative to timing of BGB-A317 and other therapies. Left panel: patient 1, right panel: patient 2.

leading to a very good partial response (VGPR). Following relapse in 2012 she was administered rituximab and bendamustine, complicated by severe rituximab infusion reactions requiring desensitization. She proceeded to consolidation with a melphalan autologous stem cell transplant. After 3 years, symptomatic relapse was associated with pancytopenia (hemoglobin 83 g/L, platelets 25x10^9/L, white cell count 1.8x10^9/L) and IgM of 37.3 g/L. CT scanning demonstrated widespread small volume lymphadenopathy with 17cm splenomegaly. Bone marrow biopsy showed a clonal lymphoplasmacytoid infiltrate accounting for 60% of cellular elements with no significant morphological dysplasia. A complex karyotype was noted including rearrangement of 1q, loss of chromosome 13 and loss of p53.

The patient was enrolled in the study (*clinicaltrials.gov identifier 02795182*) and initially started on BGB-3111, with the first dose of BGB-A317 seven days later. During the first three weeks of therapy she required granulocyte colony stimulating factor and red cell and platelet transfusions for disease related pancytopenia, and the BTK inhibitor was withheld for 27 days because of thrombocytopenia.

Fourteen days after the PD-1 antibody was administered she developed a hemolytic transfusion reaction during a red cell transfusion, with hemoglobinuria and elevation of biochemical markers of hemolysis (bilirubin 34 µmol/L, LDH 607 U/L, haptoglobin <0.2 g/L). The blood film was unremarkable, DAT was negative and repeat crossmatch of transfused units was negative by indirect antiglobulin test. An eluate from the patient's red cells gave no reaction when tested against a panel of red cell antigens.

Ongoing hemolysis of transfused (total 20 units) and native red cells necessitated administration of 1 mg/kg of oral prednisolone daily and 2 g/kg of intravenous immunoglobulin, with minimal effect. Hemolysis was accompanied by profound reticulocytopenia, with the reticulocyte nadir coinciding with the time of most severe hemolysis. The patient had pre-existing allo-antibodies (anti-E, anti-Cw, anti-Jka, anti-Kpa) but never developed a positive DAT. A repeat bone marrow biopsy was performed which demonstrated ongoing involvement by WM. Flow cytometric analysis of the lymphocyte compartment showed that the B cells (42% of lymphocytes) were clonal with kappa light chain restriction. The T cells had a CD4:CD8 ratio of 1:2.1 and there were very few NK cells.

Life-threatening uncontrolled hemolytic anemia (peak LDH 1544 U/L, nadir hemoglobin 44 g/L) prompted further immunosuppressive therapy: cyclophosphamide (750 mg/m2) and pulse IV methylprednisone (1g daily for 3 days) were administered approximately three weeks after the onset of hemolysis. BGB-3111 had been originally withheld due to thrombocytopenia, but was reinitiated under the assumption that prior BGB-A317 was the more likely cause. These measures were effective and biochemical and clinical markers of hemolytic anemia resolved within 3 weeks. The prednisone was successfully weaned and the patient has been transfusion independent for 9 months.

At the time of analysis, the patient's WM was responding to therapy, with IgM <1 g/L (VGPR) and improved thrombocytopenia, no longer requiring regular platelet transfusions. Notably, a brief cessation of BGB-3111 for a subsequent medical procedure resulted in a transient drop in hemoglobin with increased LDH and mild reticulocytopenia, which resolved with re-initiation of the medication.

Warm autoimmune hemolytic anemia has previously been reported as a rare complication of immune checkpoint inhibitors.[7-13] The two patients we describe developed a unique syndrome of acute hemolytic transfusion reactions which progressed to DAT-negative hemolytic anemia and reticulocytopenia. Neither patient had a previous history of hemolytic anemia or cold agglutinin disease. The profound reticulocytopenia and marked erythroid hypoplasia suggest that erythroid precursors may have been a predominant target of the immune attack, which may account for the persistently negative DAT. Red cell aplasia due to anti-PD-1 agents has been described once previously.[13]

Despite some atypical features, the adverse events experienced by both patients likely represent autoimmune complications of the PD-1 antibody. There was a clear temporal relationship between the administration of BGB-A317 and the hemolysis, which initially occurred 28 and 14 days after the first dose. Subsequent resolution of the syndrome took 80 and 55 days from the final dose of BGB-A317, equivalent to 5 times the estimated elimination half-life of 11 to 17 days.[14] The response to glucocorticoids and cyclophosphamide also support an immune mechanism.

No patient with a solid organ neoplasm or another lymphoid malignancy exposed to BGB-A317 has developed a similar reaction to these two patients, who were the first worldwide with WM to receive the agent. These observations suggest an integral pathophysiological role of WM, which is well known to be associated with immune dysregulation and autoimmune phenomena.[15] Additionally, WM and related indolent lymphoproliferative diseases have a well described association with autoimmune hemolytic anemia, although typically with a positive DAT.[15] We hypothesize that in both patients, control of the underlying lymphoma by BGB-3111 contributed to resolution of the hemolysis. The recrudescence of hemolysis in Patient 2 during a brief interruption of BGB-3111 supports this hypothesis.

Lymphoproliferative disorders such as WM involve complex aberrations of immune function and inhibition not limited to the malignant clone. The introduction of therapeutic agents harnessing the immune system into this patient population should be approached with caution, as demonstrated by the unexpected and severe adverse events suffered by these two patients.

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