LETTER TO THE EDITOR

Refractory/relapse thrombocytopenia in a patient with Evans’ syndrome successfully treated with zanubrutinib

BACKGROUND

Evans’ syndrome (ES) was first described by Evans in 1951 and is a rare autoimmune disorder that is defined as the concomitant or sequential occurrence of Coombs positive immune thrombocytopenia (ITP) and autoimmune haemolytic anaemia (AIHA).1 The clinical manifestations of ES include anaemia, bleeding, jaundice, hepatosplenomegaly and haemoglobinuria. Primary ES represents up to 30% of cases and its mortality rate is high. When AIHA and ITP occur concomitantly or sequentially, the diagnosis process must rule out underlying pathological conditions such as systemic lupus erythematosus (SLE), infections, primary immune deficiencies, thrombotic microangiopathies (TMA), anaemia complicating ITP, vitamin deficiencies, haematological malignancies, myelodysplastic syndromes (MDS) and paroxysmal nocturnal haemoglobinuria (PNH).2 The identification of primary and secondary ES can play a vital role in its management and prognosis. Because of the rarity of the disease, the treatment of ES is mostly extrapolated from what is recommended for isolated-autoimmune-cytopenia (ITP and AIHA), mostly relying on corticosteroids, rituximab, splenectomy, thrombopoietin receptor agonist (TPO-RAs), erythropoietin, immunosuppressants and supportive therapies. Despite recent therapeutic advances, responses to existing options are often temporary and durable remission remains elusive. Many patients go through a prolonged course and become relapsed/refractory to a series of different interventions.3 In this study, we describe the first successful case of zanubrutinib for the treatment of ES in a 15-year-old Chinese girl with severe refractory/relapsed thrombocytopenia.

CASE

A 15-year-old Chinese girl with repeated skin petechiae and ecchymosis for more than 15 months, was admitted to our hospital with worsened symptoms for 1 week in January 2021. On 30 August 2019, she had gone to a local hospital with petechiae seen on the trunk after upper respiratory tract infection. Initial work-up showed only severe thrombocytopenia (2 × 10⁹/L). A bone marrow smear and flow cytometry identified ITP. According to the latest diagnostic criteria and treatment guidelines for ITP, she was treated with high-dose dexamethasone (40mg/day) for 4 days.4 Following this, there was a transient, slight increase in platelet count to 32 × 10⁹/L.

On 14 September 2019, the patient experienced a flare and went to a haematological hospital. A full blood count showed mild anaemia (haemoglobin 114 g/L) and thrombocytopenia (7 × 10⁹/L). Bone marrow histopathological findings showed megakaryocytic hyperplasia with immaturity, no significant dysplasia or increased blasts and no evidence of metastatic carcinoma, lymphoma or granulomas. MDS-associated exome sequencing analysis did not identify a pathogenic gene mutation. Flow cytometry showed no evidence of lymphoproliferative disorder or myeloid neoplasm. Furthermore, tests for secondary thrombocytopenia were negative for antinuclear antibody, antineutrophil cytoplasmic antibody, extractable nuclear antigen, anticardiolipin antibody and lupus anticoagulant. Viral infections known to trigger ITP (Epstein–Barr virus [EBV], seasonal influenza, adenovirus, hepatitis C, hepatitis B, human immunodeficiency virus, varicella zoster virus and cytomegalovirus [CMV]) were excluded through serological tests. Her clinical examination and laboratory findings were consistent

Abbreviations: AIC, autoimmune cytopenias; AIHA, autoimmune haemolytic anaemia; ANC, Absolute neutrophil count; BCR, B cell receptor; BID, bis in die/twice day; BTK, Bruton’s Tyrosine Kinase; BTKis, Inhibitors of BTK; CLL, chronic lymphocytic leukaemia; CMV, cytomegalovirus; CT, computed tomography; EBV, Epstein–Barr virus; ES, Evans’ syndrome; Hb, Haemoglobin; HD-DXM, high-dose dexamethasone; IH, hypodermic injection; IVIG, intravenous immunoglobulin; ITP, Immune thrombocytopenia; MCL, mantle cell lymphoma; MDS, myelodysplastic syndromes; PDN, Prednisolone; PLT, Platelets; PNH, paroxysmal nocturnal haemoglobinuria; QD, quaque die/every day; rhTPO, Recombinant human thrombopoietin; SLE, Systemic lupus erythematosus; SYK, Spleen tyrosine kinase; TCM, traditional Chinese medicine; TMA, thrombotic microangiopathies; TPO-RA, Thrombopoietin receptor agonist.

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with the diagnosis of ITP. Her platelet count showed no improvement after further treatment with high-dose dexamethasone (HD-DXM) again. Treatment with recombinant human thrombopoietin (rhTPO) for 11 days resulted in a rapid recovery in platelet count \((209 \times 10^9/L)\). She took only traditional Chinese medicine (TCM) after being discharged from the hospital, resulting in a durable response\(^5\) (Table S1).

On 10 July 2020, the patient presented with epistaxis, melena, petechiae, bruises, haematuria and uterine bleeding again after a urinary tract infection. Laboratory studies revealed severe thrombocytopenia \((0 \times 10^9/L)\) and moderate-to-severe anaemia \((53\text{ g/L}~90\text{ g/L})\) with a normal white blood cell count and coagulation. A Coombs’ test was positive, leading to the diagnosis of Evans’ syndrome. She experienced several severe flares over the next 5 months and failed to respond to multiple drugs including methylprednisolone: 180mg/twice daily for 3 days and then 40mg/day; rhTPO: 15000 unit/day; initially, eltrombopag: 25mg/day for 8 days and then 75mg/day over 2 months; 1g/kg of intravenous immunoglobulin (IVIG) over 2 days; another round of HD-DXM; two doses of rituximab: 300mg/week; cyclosporine: 125mg twice daily \((4.0\text{ mg/kg})\) and azathioprine: 50mg/day (Table S1).

In November 2020, the patient developed haemolysis, persistent haematuria, uterine bleeding with headache; brain computed tomography (CT) showed subtle subarachnoid haemorrhage. Fundoscopy showed mottled haemorrhage around the optic disc of the left eye and massive haemorrhage under the internal limiting membrane in the macular region. Symptoms continued to worsen, and Doppler ultrasound of left eye showed a vitreous haemorrhage that resulted in blindness in the left eye. After multidrug therapy and platelet and erythrocyte transfusion, she showed transient improvement in the tendency to bleed, but her thrombocytopenia persisted (Table S1).

Due to further deterioration of bleeding and haemolytic symptoms, the patient was transferred to our hospital on 2 January 2021. Physical examination showed that she had severe anaemia, moderate scleral jaundice, systemic oedema and bleeding. Granulocyte and erythrocyte PNH clones were negative. No signs of haematological malignancies or lymphoproliferative disorders were detected through flow cytometry and whole-body enhanced CT. Bone marrow smear and histopathological findings still manifested megakaryocytic hyperplasia with immaturity. Genetic testing for inherited blood disorders was negative. A direct antiglobulin test (DAT) and indirect antiglobulin test found C3 antibodies. As a result of its availability as a referral test in 2019, platelet autoantibody testing was being performed in patients when ITP was suspected. All platelet autoantibody tests were performed by the KingMed Diagnostics (Zhengzhou, China) using the Pak Auto method with GTI PakAuto assay kit.\(^6\) The patient’s antiplatelet antibodies showed positive GPIIb/IIa in circulating plasma. Her clinical examination and medical history were still consistent with the diagnosis of ES. She was treated with multidrug therapy including HD-DXM, rhTPO, avatrombopag, eltrombopag, 0.4g/kg of IVIG over 5 days, splenectomy and sirolimus. During this period, the patient suffered severe bleeding with aggravation of haemolysis on several occasions. The patient’s condition was improved by active blood transfusion and anti-infection and anti-haemolysis treatment, but her platelets did not recover significantly. As a result of her poor response to the above-mentioned therapeutic interventions, she was prescribed zanubrutinib \(160\text{mg twice daily}\) on 15 March 2021. After 2 months, a dramatic response occurred, with platelet counts of \(48 \times 10^9/L\) and haemoglobin returning to normal. After 6 months, she achieved platelet count greater than \(100 \times 10^9/L\). Zanubrutinib was gradually tapered and discontinued due to hair loss and affordability, but the patient still experienced remission\(^5\) (Figure 1).

There were no obvious adverse events during the application. The proportion of CD19-positive B lymphocytes in the lymphocytes was 9.40% prior to zanubrutinib, whereas it decreased to 2.76% after 8 months. She still had positive antiplatelet antibodies and C3 antibodies after 21 months. In addition, we re-examined MYD88 L265P gene mutations and histopathological characteristics in the resected spleen tissue. The histopathology and immunohistochemistry of the spleen showed normal B cells and macrophages (Figure 2A, C, Figure S1). Sanger sequencing confirmed MYD88 L265P wild type (Figure 2B).

**DISCUSSION**

The pathophysiology of ES remains unknown, but it is recognised as a condition of immune dysregulation.\(^7\) ES is usually more challenging than ITP and has a higher mortality rate. Many cases are idiopathic, as the case presented here. Although there is no standard method to diagnose ES through laboratory tests, patients should be systematically screened for underlying or associated diseases. The majority of treatments for ES are empirical, without adequate evidence, and treatment strategies differ from centre to centre. The management of ES-ITP is based on the traditional ITP treatment strategies. Despite significant advances in the therapeutic management of ITP, it remains challenging for some patients to achieve durable remission.

In recent years, advances in understanding the pathogenesis of immune thrombocytopenia have opened up the possibility of therapeutic interventions targeting different pathways involving peripheral destruction of platelets or inappropriate myelopoiesis.\(^8\) It is well known that the B cell receptor (BCR) plays an essential role in the development and function of B cells, controlling the activation of B lymphocytes and their subsequent differentiation into antibody secreting plasm cells and memory B cells.\(^9\) Bruton tyrosine kinase (BTK) is a critical immune signalling element downstream of BCR and an essential intracellular signalling enzyme. It is also widely expressed in many cells.
and plays a critical role in B-cell maturation, antibody production, and Fcγ receptor-mediated signalling pathways. Several pathogenetic mechanisms in autoimmune diseases are implicated in the treatment regimens targeting BCR pathways. Hampel found that ibrutinib had a beneficial effect in autoimmune cytopenias (AICs) related to chronic lymphocytic leukaemia (CLL). In his study, 29 out of 193 patients treated with ibrutinib for CLL had an AIC before treatment, including eight patients with ITP and five with Evans’ syndrome. As a result, there was no worsening of AIC. In fact, 42% of treatments for AIC were reduced, while 25% were discontinued. As BTK inhibition has the potential to reduce Fcγ receptor-mediated macrophage function and autoantibody production, it may provide a new treatment option for patients with AICs, including ITP and AIHA. Thus BTK inhibitors (BTKis) become interesting target interventions in AICs, inhibiting the phagocytosis of platelets and erythrocytes by splenic macrophages. Currently approved BTKis are irreversible covalent inhibitors and have changed the management and clinical history of patients with B-cell malignancies. However, accumulating evidence shows that selective BTK inhibitors have the potential to target multiple immune-mediated diseases. Novel, less toxic and more specific BTKis could revolutionise the treatment of a large number of immune-mediated diseases with unmet treatment needs. Rilzabrutinib, an oral, reversible, covalent molecule highly selective inhibitor of BTK, could diminish platelet loss and was associated with only low-grade toxic effects across all the dose levels tested, in alignment with early clinical studies involving healthy volunteers and a preclinical study. According to the data in preclinical and clinical studies, it works via inhibition of autoantibody/Fc-gamma receptor signalling in splenic macrophages and affecting autoantibody generation through effects on B-cell activation. The preliminary results of a phase 1/2 study evaluating rilzabrutinib therapy in 60 previously treated chronic ITP patients showed promising results. In the study, heavily pretreated ITP patients, with a lack of response to prior ITP interventions, showed significant platelet responses with rilzabrutinib and maintained responses for a prolonged period of time. The drug was well tolerated, whether given as a monotherapy or with allowed concomitant ITP therapy. The phase 3 study assessing the efficacy and tolerance of rilzabrutinib in ITP is still ongoing (NCT04562766).

Spleen tyrosine kinase (Syk) is implicated in the B-cell immunopathogenesis of immune-mediated diseases and is also a potential therapeutic target. Fostamatinib, a potent small-molecule inhibitor of Syk, is an effective second-line therapy in patients with ITP. Unfortunately, we are not able to acquire fostamatinib in China. As rilzabrutinib and acalabrutinib are also not available in China, we attempted to use our available zanubrutinib. This drug was granted expedited approval by the USA and Chinese drug regulatory authorities for the treatment of mantle cell lymphoma. Zanubrutinib is a second-generation, selective covalent inhibitor of BTK and the first investigational new drug in China, thus, it is more available and cheaper than ibrutinib for our patient. We used a dose of 160mg twice daily, as recommended for the treatment of lymphoma. Our patient who failed to respond to splenectomy or other interventions finally responded to zanubrutinib, which produced a dramatic and lasting recovery without severe adverse effects. We detected a decrease in the proportion of B lymphocytes after remission, but anti-platelet antibodies and C3 antibodies were not cleared, proving that zanubrutinib may work by targeting B cells. There is a phase 2 clinical trial investigating zanubrutinib for primary ITP (NCT03395210) and we hope that it will provide more interesting discoveries. This is the first case of using zanubrutinib in refractory/relapse Evans’
FIGURE 2  (A) Splenectomy biopsy showing dilated splenic sinuses congested with spleen tissue (haematoxylin and eosin staining). (B) MYD88 L265P wild type detected by Sanger sequencing (wireframe). (C) Immunochemical staining of CD68 in spleen
syndrome. It might be possible to consider zanubrutinib for ES-ITP as a pathophysiology-guided, compassionate, off-label treatment targeting underlying disease mechanisms of platelet destruction. Although the data are limited, they might provide a reasonable paradigm for emergency repurposing of this drug in ES. However, we need more, well-designed fundamental and clinical studies to investigate a broader application of zanubrutinib in ES treatment.

KEYWORDS
autoimmune haemolytic anaemia, Bruton’s tyrosine kinase inhibitor, Evans’ syndrome, immune thrombocytopenia, zanubrutinib

AUTHOR CONTRIBUTIONS
Mengjuan L and Liu L designed, researched, analysed the data and wrote the paper. Bingjie Ding, Ao Xia, Yu Han, Yongping Song and Xuewen Song researched and analysed the data. Xudong Wei and Hu Zhou designed researched, analysed the data and revised the paper critically. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The data that supports the findings of this study are available in the supplementary material of this article.

ETHICAL APPROVAL
The study was approved by the appropriate ethics review boards. Its clinical trial registration number is ChiCTR220060868.

CONSENT FOR PUBLICATION
Verbal consent was obtained from the patient and her parents for their anonymised individual data to be published in this article.

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**SUPPORTING INFORMATION**

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