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Case Report

Rapid and sustained response to luspatercept and eltrombopag combined treatment in one case of clonal cytopenias of undetermined significance with prior failure to cyclosporin and androgen therapy: a case report

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***Abstract*:** Clonal cytopenia of undetermined significance (CCUS) has the characteristics of high-risk transformation into myelodysplastic syndromes. At present, there are few effective treatments for CCUS, and there is no consensus or evidence-based recommendation. We present a case demonstrating a rapid, significant and sustained response to combined treatment with luspatercept and eltrombopag, following the failure of cyclosporin and androgen therapy. Even after discontinuing luspatercept for 10 months, trilineage haematopoiesis remained normal with the use of cyclosporin and other haematopoietic stimulants. This case suggests that the inhibition of transforming growth factor-β could potentially have an immunomodulatory effect, thereby promoting the recovery of haematopoietic function. Luspatercept, along with Acalabrutinib or Cyclosporine, may synergistically stimulate haematopoiesis.

Keywords: case report, clonal cytopenias of undetermined significance, eltrombopag, luspatercept, rapid and sustained remission

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Introduction

Clonal cytopenias of undetermined significance (CCUS) are defined by the presence of unilineage or multilineage cytopenias, along with the detection of a clonal karyotypic abnormality and/or somatic mutations in genes involved in myeloid neoplasms, in the absence of marrow dysplasia or other diagnostic criteria for myeloid neoplasms. CCUS is considered a precursor condition with an elevated risk to progress into myelodysplastic syndromes (MDS) in subsequent 5 years following diagnosis compared with idiopathic cytopenias of undetermined significance (ICUS).[1] At present, there is a lack of effective evidence-based treatment for CCUS, and the treatment options include demethylating drugs, immunosuppressants, corticosteroids, transplantation and so on, but the effects vary.[2,3] Now we report a case of rapid, significant and sustained response to combined treatment with luspatercept and eltrombopag following the failure of cyclosporin and androgen therapy.

Case presentation

A female who was 53 years old, was admitted on 11 January 2022 for complaints of 1 month's fatigue and 2 days of gingival haemorrhage, but without any history of relevant diseases. In physical examination no swollen lymph node,

hepatomegaly or splenomegaly was palpable, bleeding spots on skin were presented. The laboratory tests including blood cell count, white blood cell (WBC) 2.45×10^9/L, haemoglobin (Hb) 56 g/L, platelet (Plt) 28×10^9/L, reticulocyte(Ret) 1.51%; folic acid and vitamin B12 were in normal reference range; no paroxysmal nocturnal haemoglobinuria (PNH) clone was detectable. The functions of liver, renal and thyroid were normal and the rheumatoimmunologic tests were negative. Erythropoietin (EPO) 346 IU/L; lymphocyte profile as $CD4^+$ 81.1%, $CD8^+$ 15.4%, CD4/CD8 ratio of 5.27, T cell receptor beta (TCR β) gene rearrangement was positive. Iliac bone marrow aspirate indicated active proliferation, granulocytic lineage was made up of 40% with left shift and erythroid lineage was 41%, neither of which had obvious dysplasia; lymphocytic cells were 18.5% and no megakaryocyte was seen. The sternal bone marrow aspirate displayed a similar profile. The bone marrow biopsy revealed that haematopoietic tissue was made up of 35% without fibrosis, normal proliferation of granulocytic and erythroid lineages, no abnormal localization of immature precursors, erythroid 'islands' were seen, the number of megakaryocytes decreased without obviously dysplasia megakaryocytes and no small megakaryocytes were found in CD41 immunohistochemistry [Figure 1(a)]. Bone marrow cells immunophenotyping showed no evidence of MDS and large granular lymphocytes, the conventional chromosome karyotype was normal. The next-generation sequencing (NGS) displayed somatic mutations of 10.8% CEBPA c.968G>C (p. Arg323Pro) and 2.43% KDM6A c.100A>G (p. Ser34Gly). Combined with the patient's relevant findings, the patient was diagnosed with CCUS.

Initially, the treatment with cyclosporin and androgen did not improve haematopoiesis significantly. Therefore, the patient relied on transfusion of red blood cells (RBCs) and platelets (per 2–3 weeks). After about 4 months of treatment (May 2022), the bone marrow aspirate displayed near-active proliferation, the myeloid cell lineages were made up of 39.6% and erythroid lineage was 27.5%, neither of which had obvious dysplasia, no megakaryocytes was seen. Bone marrow biopsy showed that haematopoietic components were made up of about 5%, with a small proportion of myelocyte, metamyelocyte, erythroblasts and small lymphocyte, lobulated nuclei megakaryocytes were seen [Figure 1(b)]. Then the EPO was introduced to stimulate the recovery of RBCs. However, the patient did not respond to EPO and remained dependent on RBC transfusion. After 9 months of treatment (October 2022) the blood cell count displayed that WBC 1.65×10^9/L, Hb 60 g/L, Plt 7×10^9/L, neutrophil (N) 32.1%, lymphocyte (L) 57.6%, mean corpuscular volume (MCV) 92.8 fl, RET 1.01%; the bone marrow biopsy showed that haematopoietic components were 5% and no megakaryocytes [Figure 1(c)], ferritin 1695 ng/mL. From 13 November 2022, luspatercept (1 mg/kg/21 day), eltrombopag (50 mg/day) and prednisone (30 mg/day) were introduced (10 months after initial treatment) for treatment.

Following the combined treatment, the patient became independent of RBC and platelet transfusions. The Hb reached 86 g/L in the third cycle of luspatercept and recovered to normal level after the fourth cycle. Subsequently, luspatercept was discontinued and haemoglobin remained at normal levels in follow-up monitoring [Figure 2(a)]. In the meanwhile, the leukocytes and platelet count were raised continuously. The WBC count reached normal reference level and the platelet count was $>20 \times 10^9$/L after the first and second cycles of luspatercept treatment, respectively. After about 22 months of treatment (November 2023), the WBC was 6.5×10^9/L, Hb 141 g/L, Plt 131×10^9/L at the last follow-up [Figure 2(b)].

About 4 months of combined treatment (March 2023), the bone marrow aspirates showed active proliferation, myeloid cell lineage was made up of 57% with myelocyte and further developed cells predominantly, erythroid cell lineage was made up of 33.5%, four granular megakaryocytes were seen in the slide. Bone marrow biopsy showed that haematopoietic components consisted of about 40% with normal myeloid and erythroid cell lineages, erythroid islands were seen, megakaryocytes count was nearly normal with lobulated nuclei predominantly [Figure 1(d)]. Ferritin fell to 1095 ng/mL, EPO fell to 7.5 IU/L.

After 7 months of combined treatment (June 2023), the lymphocyte profiling showed $CD4^+$ made up of 67.5%, $CD8^+$ of 28.1%, CD4/CD8 ratio 2.4, peripheral blood cell TCR B gene rearrangement negative; NGS displayed that the

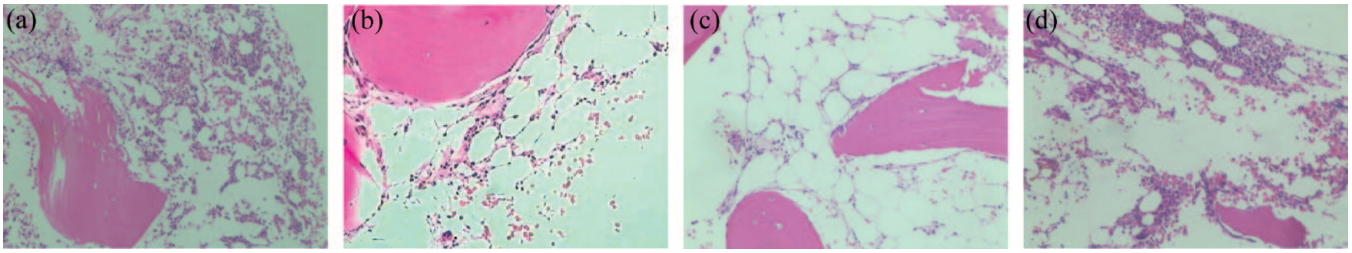

Figure 1. Bone marrow pathology biopsies of patients at different times. (a) 2022/01. (b) 2022/05. (c). 2022/10. (d) 2023/03.

somatic mutation clone *CEBPA* and *KDM6A* were not detectable, three mutations on *ASXL1* geneweredetectedas8.03%ofc.3584_3585delinsC (p. Gln1195fs), 2.09% of c.1772dup (p. Tyr591fs) and 7.32% of c.1762C>T (p.Gln588*).

Discussion

The diagnostic criteria for CCUS include decreased peripheral cytopenia with blast cells <5%, but dysplasia <10%, accompanied with one or more MDS-related gene mutations.[4] There is no datum of incidence of CCUS available yet, however, up to 75% CCUS patients would progress into myeloid malignancy in subsequent 5 years,[1] still the effective treatment for CCUS is rare and no consensus or evidence-based recommendations are available now. This patient had pancytopenia and mutations of *CEBPA* and *KMD6A* gene, without definitive dysplasia haematopoiesis, therefore, it was diagnosed as CCUS. Following diagnosis, the patient was treated with cyclosporin, androgen and EPO, but no obvious response and progress to haematopoietic failure. The patient cannot afford the allogeneic bone marrow transplantation. After the combined treatment with luspatercept and eltrombopag from November 2022 on, the counts of peripheral blood cells were increased rapidly, significantly and sustainedly.

Luspatercept could act as a transforming growth factor-β (TGF-β) superfamily ligand trap to neutralize the inhibitory effects of GDF8/11 and downstream SMAD2/3 pathway in multiple stages of erythropoiesis.[5,6] In a phase III trial (MEDALIST), the luspatercept could ameliorate the anaemia relying on regular RBC transfusion in low-risk MDS, with ring sideroblast (MDS-RS) or with *SF3B1* gene mutations, the granulocytes and platelet count were improved too.[7]

TGF-β is an important regulator of adaptive immunity and participates in regulation of the activation and function of various cells including T cells and haematopoietic stem cells.[8,9] In a TGF-βRII deficiency mice model (similar effects with TGF-β blockade), early-stage T cell development is normal but less naïve $CD4^+$ T cells in peripheral, partly due to more mature T cell death.[10] The neutralization of TGF-β *in vivo* could enhance the haematopoietic stem cell release from quiescence.[11] Luspatercept could act as a TGF-β superfamily ligand trap in the bone marrow niche and enhance the recovery of ineffective haematopoiesis.[12] At diagnosis, the patient also showed T cell dysfunction as increased $CD4^+$ cells and TCR B gene rearrangement positive, after combined treatment with luspatercept and eltrombopag, the CD4/CD8 ratio fell back into normal level, TCR B rearrangement turned negative. It was quite possible the remedy of T cell function and turned over of TCR B rearrangement could be partly explained by luspatercept treatment.

Eltrombopag acted as a thrombopoietin (TPO) receptor agonist and has been used in treatment of immune thrombocytopenia, aplastic anaemia (AA) and low-risk MDS. Besides, eltrombopag could stimulate the proliferation of haematopoietic stem cells and enhance the recovery of haematopoiesis *via* immunoregulatory effects.[13,14] Large dose eltrombopag (50–150 mg/day) could effectively enhance the recovery of trilineage haematopoiesis with a response rate of 30–40% in low-risk MDS patients.[15–17] At the median follow-up, 16 months (range: 9–42 months), some MDS patients reached robust response (Plt $>50\times10^9$/L, Hb >100 g/L

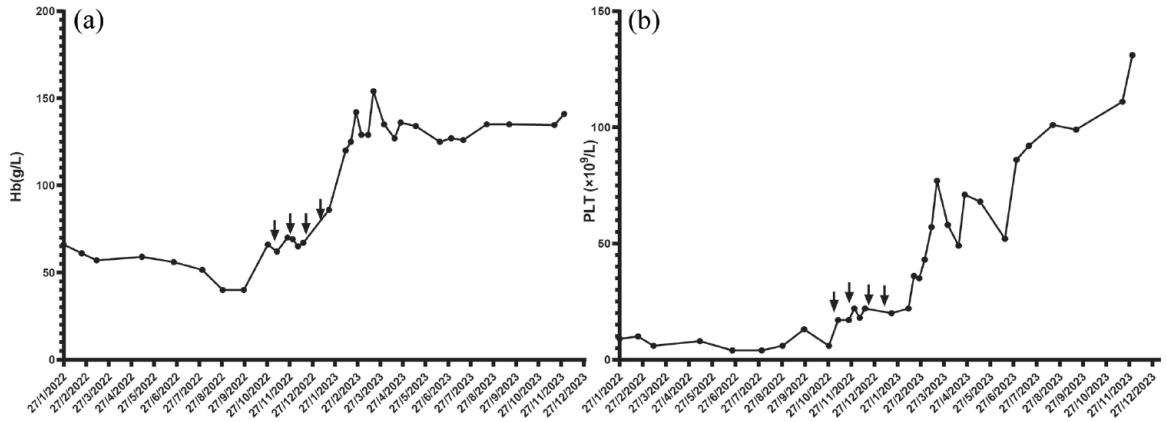

Figure 2. Changes in Hb and Plt after treatment with combined luspatercept and eltrombopag. (a:Hb;b:PLT) ↓, treatment with luspatercept; Hb, haemoglobin; Plt, platelet.

and N $>1.0\times10^9$/L), in addition, the elevated TPO and PNH clone could predict the treatment response of eltrombopag.[17] According to these criteria, this CCUS case achieved robust response following combined treatment.

Quite soon after the combined treatment of luspatercept and eltrombopag, the patient turned out to be transfusion independent both of RBCs and platelets, in the third cycle of luspatercept treatment, Hb and Plt responded rapidly. In the fourth cycle, Hb rebounded back to normal levels. Currently, the RBC retained normal even after withholding the luspatercept for 10 months. The eltrombopag, cyclosporin and androgen were still used as maintained treatment now, WBC, Hb and Plt remained normal. Bone marrow biopsy showed normal trilineage proportions. This case of CCUS demonstrated rapid, significant and sustained response to combined treatment of luspatercept and eltrombopag after treatment failure with cyclosporine, androgen and EPO, the postulated mechanism may include both the modulation of luspatercept on multiple levels of functions including TGF-β signalling, haematopoietic stem cell and its niche, immune function, especially CD4+ T cells and stimulating effects of eltrombopag on haematopoietic stem cells. Whether the luspatercept and eltrombopag could act synergistically or enhance the roles of immunosuppressant agents such as cyclosporin remained to be further investigated. Although Mathieu *et al.*[18] found that luspatercept did not modify variant allele frequencies both *in vitro* and in patient-derived xenografts (PDX) model using primary samples of MDS patients. In our patient, the somatic mutations evolved during the combined treatment from *CEBPA* and *KDM6A* genes to low-frequency mutations on *ASXL1* gene, which is a genetic mutation with poor prognosis in MDS. In a phase II, randomized, placebo-controlled clinical trial, single-agent eltrombopag was found to be effective in promoting platelet and RBC recovery in low-risk MDS, with no evidence of disease progression to acute myeloid leukemia (AML). However, the article did not report whether it led to the evolution of gene mutations.[19] Nevertheless, new mutations were observed in patients with relapsed severe AA treated with eltrombopag.[20] Furthermore, the MEDALIST Trial of Luspatercept did not demonstrate that Luspatercept significantly increased the clonal evolution of the gene mutation.[21] Consequently, the reasons for clonal evolution remain unclear, and the consequence of emerging mutations in the *ASXL1* gene should be monitored closely.

Conclusion

CCUS has the characteristics of high-risk transformation to MDS. Currently, there is no consensus or evidence-based treatment recommendations for patients with severe haemocytopenia. We herein report a case of rapid, significant and sustained response to combined treatment with luspatercept and eltrombopag following the failure of cyclosporin and androgen therapy. Even after the discontinuation of luspatercept for 10 months, the trilineage haematopoiesis remained normal with

cyclosporin and other stimulating haematopoietic drugs. This case also posed an interesting question, whether the inhibition of TGF-β could exert immunoregulatory effects to enhance the recovery of haematopoiesis, whether the luspatercept and eltrombopag or cyclosporin could act synergistically in stimulating haematopoiesis, which is worth doing further investigation.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The patient has provided written informed consent for the publication of data concerning the case presentation included in this review article.

Author contributions

Jing Xu: Conceptualization; Data curation; Methodology; Writing – original draft.

Yixin Yan: Conceptualization; Formal analysis; Writing – original draft.

Siwen Zong: Investigation; Visualization.

Wencan Ye: Data curation; Resources.

Jifu Zheng: Data curation.

Chao Min: Investigation; Visualization.

Qingming Wang: Conceptualization; Writing – review & editing.

Zhenjiang Li: Conceptualization; Funding acquisition; Investigation; Supervision; Writing – review & editing.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

Not applicable.

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