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Long-Term Patient-Customized Therapy for a Pathogenic *EPO* Mutation

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Abstract

Background: Recent advances in genomics have enabled the successful identification of a number of rare pathogenic mutations. Uncovering these mutations is essential as the first step towards devising a cure for the often debilitating and life-limiting diseases arising from them. For many of these mutations targeted agents do not yet exist. Here, we describe the case of a patient who has a novel pathogenic mutation in the erythropoietin (*EPO*) gene, which is essential for normal erythropoiesis, and who presented with a profound hypoplastic anemia.

Methods—The patient aged 5 months, was started on recombinant erythropoietin, at a standard dose of 500 units (50 U/kg) and subsequently 800 units three time weekly and her blood counts were monitored over 4 years.

Findings—A prompt response to the recombinant erythropoietin was found with an increase in hemoglobin levels to 12.8 g/dL and increase in red cell count to 4.89×10^6/uL. The patient became

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Author Contributions

Vijay G Sankaran: Conceptualization, Methodology, Reviewing and Editing, Supervision. Ayesha Ejaz: Writing - Original Draft, Writing - Reviewing & Editing. Alper Ozcan: Data Curation, Writing - Reviewing & Editing. Ekrem Unal: Data Curation, Writing - Reviewing & Editing. Musa Karakukcu: Data Curation, Writing - Reviewing & Editing.

Disclosure Statement

The authors declare that they have no conflict of interest.

transfusion independent. The therapy enabled the patient to maintain a hemoglobin level in the normal range without any adverse effects and with no requirement for further blood transfusions

Conclusions—Patient-customized therapies can be highly effective in the treatment of rare genetic disorders and for many of these disorders effective treatment may already exist in the clinical domain, as described for the patient in this report.

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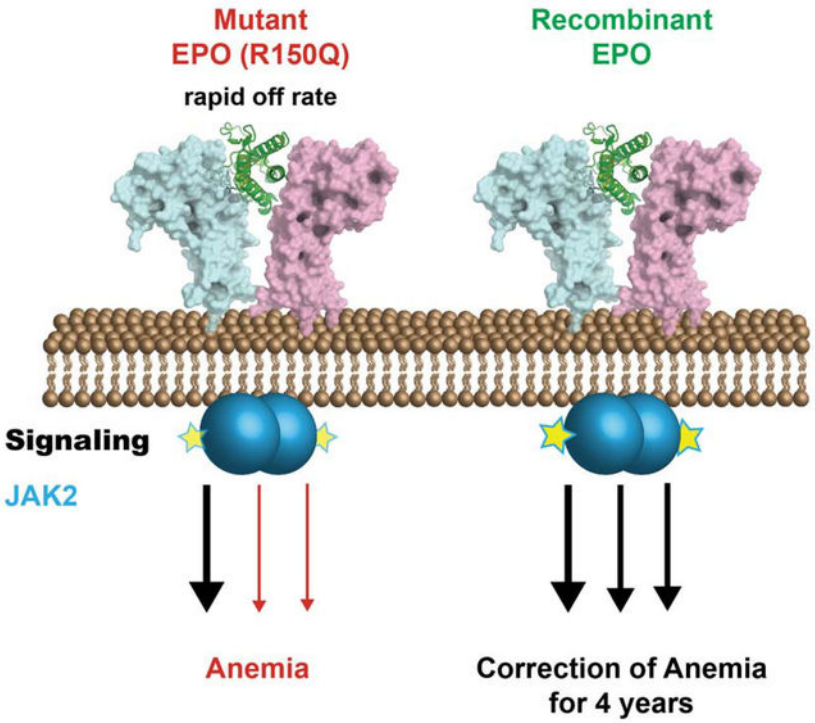
Graphical Abstract



eTOC blurb:

Ejaz *et al* describe the long-term outcome of a patient with a rare *EPO* mutation treated with recombinant erythropoietin. The case highlights the importance of undertaking genomic interrogation of rare inherited disorders as novel mutations may be found, leading to the identification of potential targeted therapies.

Introduction:

Diagnosis of rare inherited disorders has been transformed by advances in sequencing technologies in the past decade. Increasing clinical availability of exome and genome sequencing has revolutionized identification of genetic causes for uncommon conditions and has opened the door to a promising new era of individualized patient therapy. Cases in the literature[1–4] illustrate the importance of genomic sequencing in identifying previously

undescribed mutations, which can potentially be targeted therapeutically. Drug discovery is, however, often a labor-intensive, and expensive path with uncertain outcomes[5]. Repurposing of a therapy, which is already in use and can be applied to target a new disease-causing mutation may therefore be the ideal strategy for these rare disorders, bypassing the need for creating a drug discovery pipeline, obtaining regulatory approval, and for addressing the safety of experimental drug usage in a very small patient population.

We previously described a family with two children who both harbored homozygous mutations in the erythropoietin gene (*EPO*)[6]. Despite having elevated circulating levels of erythropoietin (EPO), both patients presented clinically with erythroid hypoplasia and profound anemia, requiring regular life-sustaining blood transfusions from infancy and, in the case of the older sibling, a bone marrow transplant for presumed marrow failure. Exome sequencing and rigorous functional follow up assays revealed a pathogenic biallelic R150Q mutation (chr7:100,320,704 G>A in hg19 genomic coordinates) in *EPO* with impaired binding kinetics of the cytokine to its receptor. This had resulted in impaired erythropoiesis in both children. The older sibling unfortunately passed away from complications of his bone marrow transplant, but, following this molecular diagnosis, his younger sister was initiated on recombinant erythropoietin treatment and we now report an extended update of her progress with this customized therapy.

Results & Discussion:

The patient began erythropoietin injections at the age of[5] months after her autosomal recessive mutation was diagnosed and characterized. Erythropoietin was initiated at a standard dose of 500 units (50 U/kg) three times weekly. Her dose was increased to 800 units three time weekly at 46 months due to increasing weight (Figure 1). Before treatment the patient was on a regular blood transfusion program and was maintaining a mean hemoglobin of 7.8 g/dL, far below the normal range for her age (11–15.5 g/dL). After starting erythropoietin, her erythropoiesis showed a profound positive response with an increase in hemoglobin levels to 12.8 g/dL and her hemoglobin continued to remain remarkably steady in the normal range for that time with no further blood transfusions needed (Figure 1). Her red cell and reticulocyte counts also responded rapidly to the recombinant cytokine injections with increase in her red cell count to 4.89×10^6/uL and steady maintenance in the normal range. Her reticulocyte count showed some fluctuation, but with no detriment to her other red cell parameters. No other significant blood count changes were noted either prior to or following treatment with recombinant erythropoietin.

Of note, the patient has not suffered any adverse consequences from the long-term use of recombinant erythropoietin. Issues that may be expected include the development of high blood pressure or overshooting of the hematocrit[7]. These have not occurred and the patient has developed normally with no other apparent health issues. Importantly, the patient has not required any transfusions since initiating erythropoietin therapy and has increased in weight with age, as would be expected in a healthy child (Figure 1D). As her weight increased, however, her hemoglobin level showed a tendency to drop and this was likely due to her outgrowing her initial dose of erythropoietin. Her dose was appropriately up-titrated based on her new weight to 800 units three times weekly and there has been a corresponding

increase in her hemoglobin and other red cell parameters (Figure 1). This is further proof of her physiological reliance on this exogenously administered cytokine to maintain effective erythropoiesis and normal blood count parameters. If the patient's hemoglobin levels had not improved with the increase in erythropoietin dose, then one concern would be the formation of antibodies targeting the recombinant cytokine, which can result in red cell aplasia[8]. Fortunately, the patient's counts recovered fully with the increased dose and therefore, clinically, there was no concern regarding antibody formation or other issues beyond insufficient dosing.

This case is noteworthy as it highlights the importance of undertaking genomic sequencing in unusual cases of congenital disorders. Potential therapeutic target sites may be uncovered and directed therapies may be available in the clinical domain that are ready for immediate use. These discoveries have the potential, as in the case of our patient and their family, to not only be life-altering, but to also be significantly life-prolonging. With the use of sequencing technology and the availability of erythropoietin as a highly effective targeted agent, the patient has avoided the significant risks of bone marrow transplantation (that would have failed knowing the etiology, as occurred in the patient's sibling) or the morbidity associated with chronic transfusion-related iron overload. Given the targeted therapy provided, the patient is expected to continue to develop normally with effective erythropoiesis and minimal disruption to her life by her treatment, which consists of 3 weekly subcutaneous injections.

Genomics plays a crucial role in the diagnosis of rare diseases and other examples exist in the literature where sequencing and discovery of the pathogenic mutation have led to the identification of targeted therapies that have been life-altering for patients such as ours with rare genetic conditions. One such example is congenital amegakaryocytic thrombocytopenia (CAMT), a rare disorder usually associated with mutations in the *MPL* gene. Sequencing and follow-up functional assays in a family with three affected members found a previously unknown homozygous mutation in the *THPO* gene[9,10]. The authors showed that it was disease-causing, resulting in impaired secretion of the thrombopoietin hormone. They were able to successfully treat the three patients with romiplostim, a recombinant thrombopoietin receptor agonist. This discovery of the *THPO* gene mutation and rapid response to romiplostim was life-changing, as CAMT usually requires allogeneic bone marrow transplantation to achieve a cure[9]. A similar case of a rare mutation being targeted by therapies already in use was described recently[11]. A neonate was found to have a homozygous variant of the *USP18* gene, which is essential for inhibiting interferon signaling by blocking downstream JAK2 activation through the removal of the ubiquitin-like ISG15 conjugates on a number of protein substrates. Identification of the mutation allowed the investigators to successfully initiate and demonstrate response to treatment with ruxolitinib, a widely used JAK2 inhibitor. While other patients with *USP18* mutations have died perinatally, this patient has continued to respond to treatment and is still alive at 3 years of age. Both cases of individuals with *THPO* and *USP18* mutations demonstrate the value of genomics for identifying potentially effective therapies, and also highlight how mutations impacting signaling pathways can be ideal targets, given the shared components between distinct pathways and availability of targeted small molecules (e.g. kinase inhibitors).

Beyond applications in hematology and immunology, genomics can aid in the diagnosis and development of patient-individualized therapies in other clinical fields. A case of a patient with a rare neurodegenerative disorder, Batten's disease, was described, who was found, using genome sequencing, to have a novel retrotransposon insertion in the *MFSD8* gene[12] resulting in missplicing of the *MFSD8* gene[12]. The identification of this missplicing mutation led to the development of a targeted antisense oligonucleotide (ASO) drug within a year, a case of true patient-customized therapy[4]. Importantly, no adverse effects were reported from this expedited drug development[12]. Although this study highlighted the exciting innovations in the field of genomics, it also illustrated how developing targeted treatments for a limited number of patients is labor-intensive and expensive, and will likely not be available for the vast majority of rare diseases in the immediate future.

These cases emphasize the value of using genomic techniques to uncover pathogenic and potentially targetable mutations. Where therapies already exist for these rare disorders, as in the case of our patient and those involving mutations in *THPO* or *USP18*, treatment can be initiated swiftly and in a molecularly-targeted manner. In other cases, patient-customized therapies can require time and extensive pre-clinical studies for therapeutic development. Ultimately as our knowledge of genomics expands and our ability to bring innovative genomic testing to the bedside improves, we will gain greater insights into underlying disease biology and we can aim to use this knowledge to better treat and cure patients with focused treatment strategies.

Limitations of study:

This study describes a single case of a patient with a very rare *EPO* mutation (only described in 2 patients, both members of the same family) and therefore a limitation of the paper is the very small sample size. Recombinant erythropoietin was found to have a sustained response in our patient with biological plausibility for the effect being solely due to administration of the exogenous cytokine; however, the evidence for the utility of recombinant erythropoietin would be strengthened if another case had been described with the same intervention and similar results. However, another similar case has not been identified or reported to date, to the best of our knowledge.

STAR methods

Resource availability

Lead Contact—Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Dr. Vijay Sankaran (sankaran@broadinstitute.org).

Materials Availability—This study did not involve the use of any reagents.

Data and Code Availability—This study did not generate any unique datasets or code.

Experimental model and subject details—A single patient harboring the rare erythropoietin mutation has been described: a female patient who was 5 months old at the

beginning of the study and 4 years old at the time of reporting. Other than discussed, no additional interventions or therapies were provided to the patient.

Method details—The patient was monitored clinically, and data collected on blood counts and response to erythropoietin in line with her clinical care. The patient was started on recombinant erythropoietin at 5 months of age at a standard dose of 500 units (50 U/kg) and subsequently 800 units three time weekly. The blood counts were monitored over 4 years using standard clinical laboratory assays, including complete blood counts.

Quantification and statistical analysis—GraphPad Prism version 8.4.3 was used for descriptive graphical analysis of data. There was no statistical analysis undertaken.

Additional resources—There are no additional resources to cite.

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Highlights:

- Genomic analysis of rare diseases can be used to uncover disease-causing mutations.
- These mutations can be targeted therapeutically.
- Targeted therapies may already exist and can be transformative.
- Long-term recombinant EPO is curative in a patient with a pathogenic *EPO* mutation.

Context and Significance:

This paper describes a patient with a very rare mutation in the gene for erythropoietin. Erythropoietin is a hormone essential for the development of normal red cells and this patient presented with severe anemia and depended on blood transfusions. Through meticulous scientific analysis the mutation was discovered and its effect on blood production revealed. The patient began treatment with recombinant erythropoietin, a therapy already used clinically for patients with acquired anemia, and this has enabled her to remain healthy and free of transfusions. This case, and others described in the literature, highlight the importance of genetic analysis to uncover rare mutations and show that in some cases effective, life-changing treatment may already be available in the clinical domain.

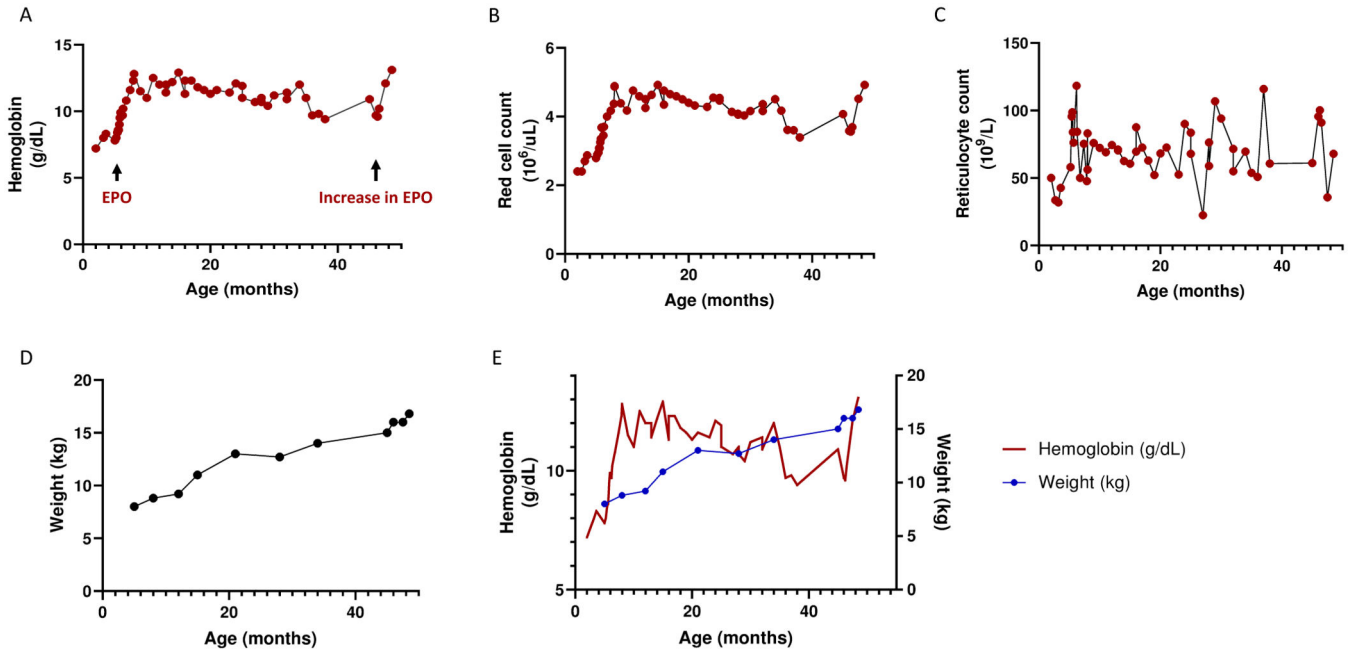

Figure 1 -

Response to erythropoietin (EPO) initiation and dose adjustment in patient with pathogenic *EPO* mutation. (A) Hemoglobin levels before and after initiation of erythropoietin at the age of 5 months. An increase in hemoglobin is seen after initiation of erythropoietin with stabilization of levels. Initiation of erythropoietin is marked with an arrow. A drop in hemoglobin was seen at 46 months and a weight-appropriate increase in dose resulted in an increase in hemoglobin, as expected. Of note, no change was seen in platelet count with erythropoietin initiation or with weight change. The patient had a normal platelet count (439,000 per microliter) before treatment initiation and maintained her platelet count in the normal range while on treatment. (B) Red cell count levels. (C) Reticulocyte count levels. (D) Increase in weight with age. (E) Drop in hemoglobin noted with increasing weight. Weight (shown in blue) increases with increasing age, while hemoglobin levels (shown in red) are shown to initially increase (due to erythropoietin), stabilize and then begin to drop as the weight continues to rise. The dose of erythropoietin was then increased at 46 months, with an increase in hemoglobin seen.