Bortezomib Treatment of Steroid-Refractory Evans Syndrome in Children

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Abbreviations

ES	Evans syndrome
AIHA	Autoimmune hemolytic anemia
ITP	Immune thrombocytopenic purpura
IST	Immunosuppressive therapy
IVIG	Intravenous immunoglobulin
PRCA	Pure red cell aplasia

Abstract

Treatment of refractory Evans syndrome (ES) remains a challenge in hematology practice. Due to rarity of this condition, evidence-based approaches are limited and often treatment choices stem from small case series or anecdotal experiences. There is mounting evidence that some patients have genetic defects that could be targeted with promising preliminary results. Here, we describe three very refractory pediatric ES cases treated on bortezomib without adverse effects. Two of the three patients had dramatical and long-lasting recovery that started following the initial doses of the drug. Clinical trials to assess bortezomib role in ES treatment are warranted.

Introduction

Evans Syndrome (ES) is a rare childhood autoimmune disorder characterized by the destruction of at least two blood cell lineages without any underlying pathological associations, often by anemia and thrombocytopenia and frequently accompanying neutropenia[1]. Autoantibodies target red blood cells, platelets, and neutrophils leading to autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP) and autoimmune neutropenia, respectively. Responses to variety of immunosuppressive therapy (IST) approaches are often temporary; many cases experience a prolonged course and become refractory to a series of different IST interventions[2,3]. Treatment of ES has been largely through trial and error-like approach with differences in management between different centers[4]. Here, we present the course of three children with refractory ES treated with Bortezomib.

Case Descriptions

Case1. The initial course of this case was previously reported[5]. A currently 15-year-old male was diagnosed with ES at 9 years of age and later developed panhypogammaglobulinemia and required regular intravenous immunoglobulin (IVIG) supplementation for the treatment of common variable immunodeficiency. He has had several lines of IST to control AIHA and ITP with initial responses followed by recurrences. He initially experienced long-lived remissions following rituximab therapy and later splenectomy. He later presented with hemolysis and was treated with bortezomib.

He received bortezomib at a dose of 1.3 mg/m^2 on days 1, 4, 8, 11, and 26 partially based on the clinical trial for refractory autoimmune cytopenias following allogeneic hematopoietic stem cell

transplantation (Clinicaltrials.gov Identifier: NCT01930253). He experienced a dramatic clinical response with immediate improvement within 4 days of the first dose providing him a remission that lasted for 22 months until he developed thrombocytopenia. Following a single dose of bortezomib at 1.3mg/m^2, he achieved platelet count greater than 100x10^9/L in 2 weeks providing an ongoing remission of 7 months so far (Figure1, Table1). He continues to have regular IVIG infusions. No genetic testing is available on this patient.

Case2. A currently 2-year-old boy presented at 6 months of age with fever, severe anemia with a hemoglobin of 2.1g/dL, reticulocytes 0.1%, absent neutrophils and mild thrombocytopenia. Coombs test and anti-neutrophil antibodies were positive, and IgM elevated at 351mg/dL (20-145). Bone marrow showed a paucity of erythroid precursors, myeloid left shift, and increased megakaryocytes consistent with pure red cell aplasia (PRCA). Investigation for known genetic causes of PRCA, hyper-IgM syndrome, and later whole exome sequencing did not identify a pathologic causative mutation.

He became refractory to various IST and remained blood transfusion-dependent due to continuing PRCA. Several episodes of infections associated with severe neutropenia requiring admission responded to filgrastim therapy. Later, platelets dropped to 1-2x10^9/L with active multisystem bleedingrequiring several admissions with further elevation in IgM to 495mg/dL. Due to continued life-threatening bleeding for months without any response to rituximab, bortezomib therapy (4 doses total) was given. After the second dose, he showed an increase in platelets; all counts normalized with reticulocytosis two weeks from

the first dose. Since he received rituximab therapy 6 weeks prior to bortezomib treatment, serum immunoglobulins and B cells were declining rapidly.He became panhypogammaglobulinemic and completely B cell depleted, so he was given IVIG supplementation. He continues to have normal counts and immunoglobulin levels with negative Coombs test a year following bortezomib therapy (Figure1, Table1).

Case3. A 15-year-old male patient presented with persistent bleeding and was found to have pancytopenia with positive Coombs test and anti-neutrophil antibodies, and elevated IgG level. He had poor response to several different therapeutic interventions. Therapy with a thrombopoietin receptor agonist, romiplostim, resulted in platelet count recovery with continuing neutropenia and mild anemia. He developed several episodes of tonsillitis and tonsillar enlargement that resolved after rituximab-biosimilar treatment. He continued to be dependent on romiplostim therapy to maintain platelet count. Therefore, he was treated with the same regimen of bortezomib. However, due to lack of response, he was restarted on romiplostim. Serum immunoglobulins and B cells were trending down significantly due to rituximab-biosimilar therapy given 8 weeks earlier that continued following bortezomib treatment with low IgM, and absent B cells; no IVIG supplementation was given. Whole exome sequencing analysis did not identify a pathologic causative mutation(Figure1, Table1). Bortezomib was tolerated well without any peripheral neuropathy or infections in all three patients.

Discussion

There are no clinical trials to validate the use of any second- or third-line therapies in steroid-refractory ES in children. Often, physicians are left to draw conclusions from case reports or series.

Steroids alone are seldom effective in providing sustained remission; thus, are considered an adjunct to other therapies. Rituximab induces complete/partial remissions in at least one cell line in approximately 76% of children.[6] Rituximab treatment carries the risk of infections, hypogammaglobulinemia, and multifocal leukoencephalopathy. Other therapies such as IVIG, mycophenolate mofetil, sirolimus, cyclosporine, and splenectomy have had variable success.[2]

Heterogeneity of treatment responses reflects variations in the underlying mechanism(s). Thus, it is likely that several different steps of the immune response may be involved in the development of ES, which has been evidenced by more recent demonstrations of various underlying mutations in immune response genes.[7] However, since autoantibodies play a terminal role in ES, B cell-plasma cell pathway has been the target of several current therapies. Inability of B cell-depleting rituximab in eliminating long-lived plasma cells lacking CD20 could be the clue in its failure in some cases. Thus, long-lived plasma cell eradication is a reasonable approach in such cases, which maybe achieved with bortezomib given its success in in multiple myeloma therapy targeting malignant plasma cells. Additionally, bortezomib has shown success in anecdotal cases of refractory ITP and ES and in-vitro evidence suggested elimination of long-lived plasma cells[5,8-10].

Two of three cases reviewed here showed a dramatic and long-lasting response to bortezomib therapy. We would like to stress three observations from our limited experience: 1. Initial response came rather fast raising the possibility of mechanisms involved beyond inhibition of autoantibody production through elimination of long-lived plasma cells, since half-life of IgG is approximately 3 weeks and such autoantibodies tend to be high-affinity likely secondary to affinity maturation over time requiring further research. 2. In line with the rapidity of response and furthermore, lasting recovery after a single dose of bortezomib following a flare in the first case, smaller and/or lesser

number of doses could be sufficient to induce responses. 3. Bortezomib has been well-tolerated without any evidence of peripheral neuropathy, the frequent side effect in patients with multiple myeloma, infections or any other adverse effects.

Increased recognition of underlying monogenic mutations in various immune response genes in ES patients, raises the possibility of new targeted therapeutic interventions that would eventually prevent autoantibody production along with control of other systemic accompanying processes.[11-12] These novel targeted therapies are being currently investigated with some promising results.[13,14] However, many patients may not have access to genetic testing, including whole exome or genome sequencing. Several cases do not have yet described genetic etiology despite testing as exemplified in second and third cases in this report. Furthermore, even with some known underlying genetic abnormalities, targeted therapies may not be currently available. We think clinical trials integrating bortezomib in ES treatment is reasonable in steroid-refractory patients, particularly, if they failed rituximab therapy after reaching complete peripheral B cell lymphodepletion. It is also prudent to have genetic testing for underlying immune dysregulation screening, if available.Patients receiving bortezomib therapy should be monitored for fatigue, weakness, peripheral neuropathy, nausea, vomiting, diarrhea, fever, constipation, thrombocytopenia and anemia,in addition to hypogammaglobulinemia.Bortezomib-responsive cases may avoid several potential adverse effects, and social and financial burden of other interventions. Furthermore, biological correlates of bortezomib-responsive and refractory cases may help understand the various mechanisms of ES.

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Figure Legend

	Medication	Duration	Response		
			Hb	Plt	ANC
Patient 1	Prednisone	3.5 months	None	Temporary	Unaffected
	Mycophenolate	3.5 months	None	None	Unaffected
	IVIG	Monthly	None	Temporary	Unaffected
	Rituximab	1 dose	Temporary	None	Unaffected
	Sirolimus	3 months	None	None	Unaffected
	Bortezomib	5 doses	Sustained	Sustained	Unaffected

	Medication	Duration	Response		
			Hb	Plt	ANC
Patient 2	Prednisone#	14 months	Temporary	None	None
	IVIG	4 months	None	None	None
	Neupogen	5 months	None	None	Temporary
	Elthrombopag	3 months	None	None	None
	Rituximab	2 doses	None	None	None
	Sirolimus	3 months	None	None	None
	Dexamethasone	2 doses	None	None	None
	Bortezomib	4 doses	Sustained	Sustained	Sustained

	Medication	Duration	Response		
			Hb	Plt	ANC
Patient 3	Romiplostim	9 months	None	Temporary	None
	Prednisone	2 month	None	Temporary	Temporary
	IVIG	1 dose	None	None	None
	Rituximab-BS	4 doses	None	Temporary	None
	Bortezomib	5 doses	None	Temporary	None
	Dexamethasone*	6 months	None	Temporary	Temporary

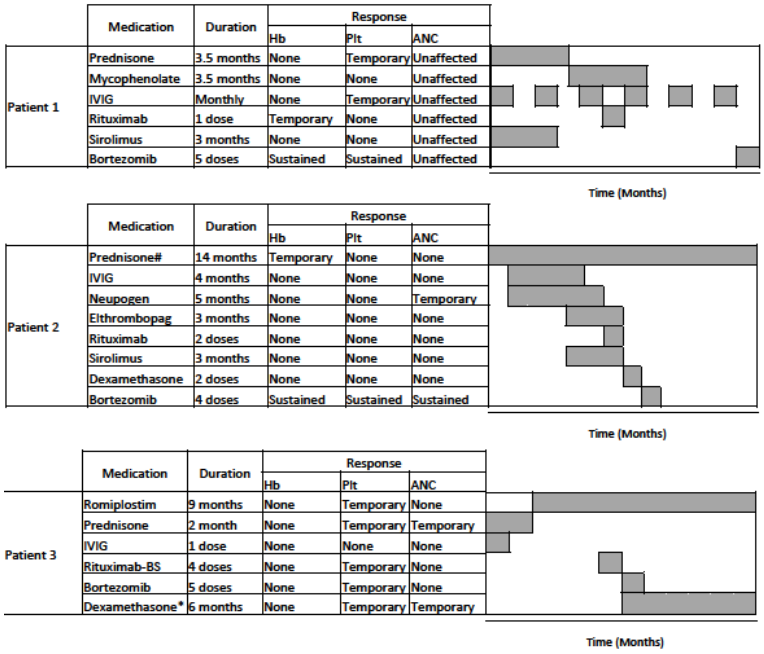

Figure1. Schematic illustration of response to various treatments in three patients presented. Therapeutic interventions used in the first case was limited to 6 moths before the first bortezomib treatment in the figure. The first patient did not have neutropenia. Abbreviation: IVIG: Intravenous immunoglobulin; BS: Biosimilar; Hb: Hemoglobin; Plt: Platelets; ANC: Absolute neutrophil count

#Prednisone has been tapered during and following bortezomib therapy

* Dexamethasone was used intermittently during episodes of severe neutropenia at a pulse dose of 20mg for 4 days

Case	Age@Dx	Case characteristics	Genetic test findings	Treatment	Current Findings
1	9 years	Hemolytic anemia Thrombocytopenia Positive Coombs test CVID	No genetic tests are available	Transfusions Steroids Rituximab Mycophenolate IVIG Sirolimus Splenectomy Bortezomib	Positive Coombs test Low IgG and IgM Low B cells Clonal T-LGL expansion

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2	**6 months**	**Thrombocytopenia** **Anemia** **Reticulocytopenia** **Neutropenia** **PRCA** **Positive Coombs test** **Positive anti-neutrophil Ab** **Elevated IgM** **Elevated IgA** **G6PD deficiency** **HbC trait** **Duffy null phenotype**	**VUS – HBB c.19G>A [Pathologic; heterozygous; consistent with HbC trait)** **VUS – ELANE c.778C>A [Heterozygous; likely to be benign]**	**Transfusions** **Steroids** **Filgrastim** **IVIG** **Filgrastim** **Eltrombopag** **Rituximab** **Sirolimus** **IV pulse MPD** **Bortezomib**	**Negative Coombs test** **Positive anti-neutrophil Ab** **Normal immunoglobulin levels** **Normal lymphocyte subsets**

3	**15 years**	**Thrombocytopenia** **Neutropenia** **Positive Coombs test** **Positive anti-neutrophil Ab** **Elevated IgG** **Elevated IgA** **Obesity** **Acanthosis nigricans**	**VUS – WAS exon 10 c.1144C>T ->p.Pro382Ser. [Maternally inherited; hemizygous deletion; not likely to be destructive]** **VUS – FLT4 exon 20 c.2810A>G -->p. Asn937Ser. [Maternally inherited; heterozygous; possibly damaging]**	**IVIG** **IV pulse MPD** **IV pulse DXM** **Oral steroids** **Rituximab-BS** **Romiplostim** **Bortezomib**	**Positive Coombs test** **Positive anti-neutrophil Ab** **Low IgM level** **Low B cells** **Clonal T-LGL expansion**

Table1. Case clinical and laboratory finding summaries

Abbreviations. Dx: Diagnosis; CVID: Common variable immunodeficiency; PRCA: Pure Red Cell Aplasia; Ab: Antibody; G6PD: Glucose 6-phosphate dehydrogenase; HbC: Hemoglobin C; VUS: Variant of uncertain significance; IVIG: Intravenous immunoglobulin; IV: Intravenous; MPD: Methylprednisolone; DXM: Dexamethasone; BS: Biosimilar; T-LGL: T-cell large granular lymphocyte