Development of red blood cell autoantibodies following treatment with checkpoint inhibitors: a new class of anti-neoplastic, immunotherapeutic agents associated with immune dysregulation

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Ipilimumab, nivolumab, and pembrolizumab represent a new class of immunotherapeutic drugs for treating patients with advanced cancer. Known as checkpoint inhibitors, these drugs act to upregulate the cellular and humoral immune response to tumor antigens by inhibiting T-cell autoregulation. As a consequence, they can be associated with immune-related adverse events (irAEs) due to loss of self-tolerance, including rare cases of immune-related cytopenias. We performed a retrospective clinical chart review, including serologic, hematology, and chemistry laboratory results, of two patients who developed red blood cell (RBC) autoantibodies during treatment with a checkpoint inhibitor. Serologic testing of blood samples from these patients during induction therapy with ipilimumab and nivolumab, respectively, showed their RBCs to be positive by the direct antiglobulin test (IgG+, C3+) and their plasma to contain panreactive RBC autoantibodies. Neither patient had evidence of hemolysis. Both patients developed an additional irAE during treatment. A literature review for patients who had developed immune-mediated cytopenia following treatment with a checkpoint inhibitor was performed. Nine other patients were reported with a hematologic irAE, including six with anemia attributable to autoimmune anemia, aplastic anemia, or pure RBC aplasia. Hematologic irAEs tend to occur early during induction therapy, often coincident with irAEs of other organs. In conclusion, checkpoint inhibitors can be associated with the development of autoantibodies, immune-mediated cytopenias, pure RBC aplasia, and aplastic anemia. Immunohematology reference laboratories should be aware of these agents when evaluating patients with advanced cancer and new-onset autoantibodies, anemia, and other cytopenias. *Immunohematology* 2017;33:15–21.

Key Words: checkpoint inhibitor, autoantibody, anemia, cytopenia

Checkpoint inhibitors are a new class of immunotherapeutic agents aimed at increasing the host immune response against tumor antigens. These include monoclonal antibodies against cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4, CD152), programmed cell death protein 1 (PD-1, CD279), and programmed cell death ligand 1 (PD-L1,

Table 1. Checkpoint inhibitors

Drug class (trade name, manufacturer)
Anti-CTLA-4
Ipilimumab (Yervoy, Bristol-Myers Squibb)
Tremelimumab (AstraZeneca, compassionate use only)
Anti-PD-1
Nivolumab (Opdivo, Bristol-Myers Squibb)
Pembrolizumab (Keytruda, Merck Sharp & Dohme)
Pidilizumab (Medivations, in clinical trials)
Anti-PD-L1
Atezolizumab (Genentech, in clinical trials)
Durvalumab (AstraZeneca, approved bladder cancer)

CTLA-4 = cytotoxic T-lymphocyte–associated antigen 4; PD-1 = programmed cell death protein 1; PD-L1 = programmed cell death ligand 1.

CD274) (Table 1). In clinical studies, checkpoint inhibitors have been shown to significantly increase survival in patients with late-stage cancer. Ipilimumab, a monoclonal antibody against CTLA-4, commonly used for patients with advanced metastatic melanoma, increases both median and overall survival, with some patients showing prolonged clinical remission for several years.[1–3] Ipilimumab has also been used successfully in patients with metastatic prostate cancer and in patients with leukemia who relapse after allogeneic stem cell transplantation.[1,3,4] Likewise, monoclonal antibodies against PD-1 have shown equally promising results in patients with advanced melanoma, lung cancer, and renal cell carcinoma.[5–8] At present, there are nearly 100 clinical trials involving checkpoint inhibitors being used for patients with cancer, including patients with lymphoma, multiple myeloma, melanoma, and cancers of the breast, colon, kidney, pancreas, ovary, prostate, lung, and head and neck.[9]

Ipilimumab and other CTLA-4 monoclonal antibodies act to increase immune responsiveness to tumor antigens by

disrupting T-cell autoregulation at the time of initial antigen presentation. Normally, T-cell activation is tightly regulated, requiring the interaction of two distinct ligand-receptor pairs: the T-cell receptor (TCR)–major histocompatibility complex (MHC) and CD28-B7.[1,5] Initially, antigen-presenting cells (APCs), such as macrophages and dendritic cells, bind tumor/ foreign antigens via the MHC and then present the bound antigen to the TCR on T-cells. This interaction of MHC-antigen with the TCR is the first step in T-cell activation. Subsequent binding between B7 on APCs and CD28 on T-cells delivers a co-stimulatory signal necessary for T-cell proliferation and cytokine secretion. T-cell activation is negatively regulated by CTLA-4, which is upregulated upon initial TCR activation and blocks the B7-CD28 co-stimulatory signal. Specifically, CTLA-4 is a high-affinity receptor for B7 and effectively outcompetes CD28 for B7 binding, thereby limiting and blunting T-cell activation. Monoclonal antibodies against CTLA-4 inhibit this self-regulation, allowing a prolonged and amplified T-cell response, enhancing both cellular and humoral immunity.[1,3]

PD-1 and PD-L1 inhibitors, on the other hand, appear to act primarily on T-effector cells present in tumor and tissues.[5,10] PD-1 is expressed on activated T-cells, whereas its ligand, PD-L1, is widely expressed on lymphohematopoietic cells, epithelial tissues, and on several cancer cell types, such as those associated with leukemia, melanoma, and carcinoma. In cancer, PD-L1/PD-1 interactions are one mechanism by which tumors escape immune surveillance with disease relapse. Like CTLA-4-CD28 signaling, the binding of tissue PD-L1 to PD-1 on activated T-cells transmits an inhibitory signal that blunts T-cell activation by promoting T-cell apoptosis and anergy.[5] PD-1 and PD-L1 antibodies block this autoregulatory pathway, promoting T-cell expansion and cytokine secretion.

Given their mechanism of action, it is not surprising that the most common adverse events associated with checkpoint inhibitors are autoimmune phenomena. Up to 70 percent of patients receiving ipilimumab are reported to have at least one immune-related adverse event (irAE).[1,3] The most common irAEs are dermatologic with rash and/or pruritus (45–65%); gastrointestinal with diarrhea, colitis, and intestinal perforation (33%); endocrine disorders due to thyroiditis, hypopituitarism, hypophysitis, and adrenal insufficiency (4.5%); and hepatic including transaminitis, autoimmune hepatitis, and hepatic failure (6%). Other less common irAEs include neurologic disorders, ocular inflammation, pancreatitis, pneumonitis, systemic vasculitis, immune cytopenias, and acquired hemophilia A.[1] Laboratory studies often show a lymphocytic infiltrate in affected organs, sometimes accompanied by circulating autoantibodies.[1,3] PD-1 inhibitors are reported to have a lower incidence of irAEs, but their irAEs can still be life-threatening.[5–8,11] IrAEs generally occur within the first few months of starting immunotherapy, with an average onset of 6 weeks.[1]

Immune cytopenia is considered a rare irAE (1%) and includes pancytopenia, immune thrombocytopenia, immune neutropenia, aplastic anemia, warm autoimmune hemolytic anemia (WAIHA), and pure red blood cell (RBC) aplasia.[1,3,4,12–16] We present two patients who developed strong warm and cold RBC autoantibodies during induction immunotherapy with ipilimumab and nivolumab, respectively. In addition, we have reviewed and summarized the clinical and laboratory findings in patients with immune-related cytopenias associated with checkpoint inhibitor therapy.

Materials and Methods

Routine ABO and D typing and antibody detection testing were performed using a gel-based analyzer (ProVue, Ortho Clinical Diagnostics, Raritan, NJ). Samples with abnormal test results were referred to the University of Michigan Immunohematology Reference Laboratory for further investigation using manual methods.

Antibody identification was performed initially by gel method (ID-MTS, Ortho Clinical Diagnostics) using commercial panel RBCs (0.8%, native, and ficin-treated) per the manufacturer's instructions. Testing for cold agglutinins was performed by the tube method (immediate spin, followed by a 15-minute room temperature incubation).[17] For adsorption studies, patient plasma was incubated with washed, enzyme-treated RBCs (ZZAP; 0.02% ficin, 0.1M dithiothreitol final concentration) at 37°C for 30 minutes, followed by centrifugation (3100g × 5 minutes) as described.[17] Adsorbed plasma was tested in parallel with unadsorbed plasma by the saline tube method, with readings after 37°C incubation (60 minutes) and after the indirect antiglobulin test (IAT) using polyspecific antihuman globulin (AHG) (rabbit polyclonal IgG, mouse monoclonal anti-C3b and anti-C3d; Ortho Clinical Diagnostics).

A direct antiglobulin test (DAT) was performed by standard tube method using polyspecific AHG (Ortho Clinical Diagnostics), monospecific anti-IgG (polyclonal, Ortho Clinical Diagnostics), anti-C3b,-C3d (monoclonal blend, ImmucorGamma, Norcross, GA), and 6 percent albumin control (Ortho Clinical Diagnostics).[17] Elution was performed on patient RBCs using an acid elution method (Gamma Elu-Kit II, ImmucorGamma). The eluate was tested against selected

native and ficin-treated RBCs by tube (30 minutes at 37°C; Anti-IgG, Ortho Clinical Diagnostics) with polyethylene glycol (PEG; GammaPeG, ImmucorGamma) enhancement.[17,18]

Extended RBC phenotyping was performed by tube method using commercial blood typing reagents against C, c, E, and e (Rh gel cards, MTS, Ortho Clinical Diagnostics); Fy^a and Fy^b (human polyclonal, Ortho Clinical Diagnostics); and K, S, s, Jk^a, and Jk^b (IgM monoclonal, ImmucorGamma). All testing was performed in parallel with antigen-positive and antigen-negative RBC controls.

Case Reports

Patient 1

The patient was a 65-year-old, group B, D− woman with metastatic melanoma to the bilateral groin, with radiologic extension into the deep perineum and multiple perineal lymph nodes (Table 2). Molecular testing of her tumor showed that it was negative for c-KIT mutations but was positive for a mutation in B-RAF (V600E), a serine/threonine protein kinase. The patient was initially treated with the targeted B-RAF inhibitor vermurafenib (Zelboraf; Genentech, South San Francisco, CA) for 4 months without clinical improvement and with radiologic evidence of disease progression. Her other medications included alprazolam, clindamycin, metronidazole, and acetaminophen/hydromorphone.

The patient subsequently underwent evaluation to start treatment with ipilimumab, an anti-CTLA-4 inhibitor. Clinical issues included ongoing bleeding from skin ulcers at the site of her bilateral groin masses, resulting in severe anemia (hemoglobin [Hb] 6 g/dL, normal 12–16 g/dL). As a result, she was transfused with 2 units of RBCs. She was seen 1 week later for her first scheduled ipilimumab infusion and was again severely anemic (Hb 6.7 g/dL) due to blood loss. She was transfused an additional 2 units of RBCs. Her antibody detection test on both occasions was negative, and there was no history of alloantibodies.

The patient was started on ipilimumab at the standard induction dose of 3 mg/kg every 21 days for four cycles. After her first dose of ipilimumab, she developed a pruritic rash on her trunk and lower leg, which was treated with over-the-counter medication. The patient was subsequently admitted 3 weeks later, just prior to her second ipilimumab dose, because of continued bleeding and possible infection of her groin masses. Her Hb on admission was 3.7 g/dL. The patient again typed as group B, D−, but her antibody detection test was now positive. A standard antibody panel (gel) identified anti-KEL1. Her extended phenotype was ce

(rr), KEL:−1, S+s+, Jk(a+b+), Fy(a+ b+). A phenotype of the four previously transfused RBC units showed that 1 unit was KEL:1. There was no laboratory evidence of a delayed hemolytic transfusion reaction: DAT and eluate were negative. Other laboratory tests showed a chronically elevated lactate dehydrogenase (460–782 IU/L, normal 129–240 IU/L), normal haptoglobin (191 mg/dL, normal 22–239 mg/dL), and normal total bilirubin (0.5 mg/dL, normal 0.2–1.2 mg/dL). A peripheral blood smear showed a mild microcytic anemia and polychromasia with no spherocytes, consistent with an increased RBC distribution width (18%; normal 11.5–15%), mildly decreased mean corpuscular volume (75.1 fL, normal 79–99 fL), and mean cell Hb concentration (27.7 g/dL, normal 32–35 g/dL). Her platelet and total white blood cell (WBC) counts were normal except for a mild absolute lymphopenia (900/μL, normal 1.2–4 K/μL). She was transfused with 4 units of KEL:−1, crossmatch-compatible RBCs with an appropriate post-transfusion response (Hb 8.0 g/dL). In addition, she received localized radiotherapy and cycle 2 of ipilimumab.

The patient returned to the clinic to receive her third cycle of ipilimumab. Her Hb was 8.5 g/dL. A new specimen showed a positive antibody detection test with both KEL:1 and KEL:−1 reagent RBCs. The patient's RBCs reacted by the DAT: polyspecific AHG (2+), anti-IgG (1+), and anti-C3 (2+) with a negative albumin control (Table 3). The patient's plasma was tested with a limited cold agglutinin cell panel, consisting of group O adult (n = 2), group B adult (n = 2), group O cord cells (n = 1), and an autocontrol; all tests were reactive at both immediate spin (1+, tube) and after 15-minute room temperature incubation (3+). A standard antibody identification panel showed strong panreactivity with 11 of 11 cells in the IAT (2+ to 3+; gel); reactivity was enhanced with ficin-treated cells (4+). An acid eluate prepared from the patient's RBCs was equally reactive with native (2+) and ficin-treated (2+) group O, D−, KEL:−1 RBCs by the PEG-IAT tube method. Because of recent transfusions, an autoadsorption was not performed. Instead, the patient's plasma was alloadsorbed four times using ZZAP-treated, group O, D− (rr) RBCs, followed by testing against a standard antibody panel by saline tube method with readings after 60-minute 37°C incubation and after the IAT using polyspecific AHG. Adsorbed plasma was reactive with only KEL:1 RBCs; no other alloantibody specificities were found. In contrast, the unadsorbed plasma control showed strong panreactivity (3+) after 37°C incubation, with variable reactivity at the IAT-AHG phase (trace to 2+). There was no laboratory evidence of hemolysis. A review of her medications showed no new medications in the last several months except ipilimumab.

Table 2. Immune-associated cytopenias in patients on checkpoint inhibitors

Reference	Age (years)/ gender	Diagnosis	Drug	Dose (mg/kg)	Number of doses	Hematologic adverse event	Onset (weeks)	Treatment	Response	Other adverse events
12	77/F	Melanoma	Ipilimumab	10	4	Pancytopenia	10	Steroids	Steroid resistant	Autoimmune hyperthyroidism
								IVIG	IVIG responsive in 8 weeks, relapse at 12 weeks	
								Erythropoietin		
								GM-CSF		
								Eltrombopag		
13	60/M	Melanoma	Ipilimumab	10	9	Pure RBC aplasia	>50	Steroids	Steroid resistant	Vitiligo Diarrhea/colitis Hypothyroidism
								IVIG	IVIG responsive	
14	68/F	Melanoma	Ipilimumab	3	3	WAIHA	~11	Steroids	Steroid sensitive	
14	49/M	Melanoma	Ipilimumab	3	3	Neutropenia	9–11	Steroids	Steroid sensitive	
								GM-CSF		
14	70/F	Melanoma	Ipilimumab	3	4	Anemia	48	Steroids	Steroid sensitive	Pruritis
						Neutropenia		GM-CSF		
3	NA/M	Prostate carcinoma	Ipilimumab	10	2	Aplastic anemia	>12	Steroids	Steroid resistant	Autoimmune hepatitis
								Cyclosporine	Cyclosporine resistant	
								ATG	ATG resistant	
4	NA	Allogeneic stem cell transplant	Ipilimumab	10	<4	Immune thrombocytopenia	<12	Steroids	Steroid sensitive	
									Resumed ipilimumab	
15	85/M	Melanoma	Ipilimumab	3	4	None	—	—	—	ANCA+
			Nivolumab	3	5	WAIHA	10	Steroids	Steroid sensitive	
16	52/F	Melanoma	Ipilimumab	NA	NA	None	—	—	—	Autoimmune hepatitis
			Pembrolizumab	NA	3	WAIHA	NA	Steroids	Steroid sensitive, relapse at 6 weeks	
						Pure RBC aplasia		IVIG	IVIG responsive	
Patient 1	65/F	Melanoma	Ipilimumab	3	2	Warm and cold autoantibodies	7	None	None	Rash/pruritis
Patient 2	66/M	Small cell carcinoma lung	Nivolumab	3	4	Warm autoantibodies	6	None	None	Arthritis

IVIG = intravenous immunoglobulin; GM-CSF = granulocyte-macrophage colony stimulating factor; RBC = red blood cell; WAIHA = warm autoimmune hemolytic anemia; NA = not available; ATG = anti-thymocyte globulin; ANCA = anti-neutrophil cytoplasmic antibody.

The patient completed induction therapy without further sequelae and did not receive steroids or other immunosuppression for any irAE. After 6 months, she was transitioned to pembrolizumab, an anti-PD-1 inhibitor (Table 1), because of disease progression. The patient tolerated pembrolizumab (2 mg every 21 days for nine cycles) with no documented irAE. A repeat DAT 1 year after receiving ipilimumab, as well as pembrolizumab, was negative. The patient died of metastatic disease 2 years after completing ipilimumab.

Patient 2

The patient was a 66-year-old man with small cell carcinoma of the lung, initially treated with cisplatin and etoposide (×2), followed by carboplatin and cisplatin (×3) and radiation (Table 2). After finding evidence of disease progression with metastasis, the patient was placed on nivolumab, an anti-PD-1 inhibitor, at 3 mg/kg every 2 weeks for 10 weeks. Within 2 weeks of his first dose, the patient developed new-onset joint swelling and pain in both hands, which was attributed to nivolumab.[19] The patient was prescribed ibuprofen (800 mg every 8 hours) and was followed clinically without further diagnostic studies. At the time of his fourth cycle, the patient complained of fatigue and shortness of breath coincident with worsening anemia (Hb 8.2 g/dL). His RBC indices, WBC count, and platelet count were within normal limits, and there was no objective evidence of hemolysis or blood loss. A WBC differential was unremarkable with a normal relative and absolute lymphocyte count.

A type and screen was ordered at the time of his fourth cycle because of symptomatic anemia (Table 3). His RBC phenotype was group A, D– (rr); his antibody detection test was positive.

His RBCs reacted by the DAT with polyspecific AHG (2+), anti-IgG (2+), and anti-C3 (2+). His plasma reacted with 11 of 11 cells at 37°C by IAT gel (2+); reactivity was enhanced by ficin (4+). The autocontrol was positive (1+). Testing his plasma with a cold panel showed no reactivity against group O cells at either immediate spin or 15-minute room temperature incubation. By saline tube method, the patient's plasma was nonreactive after 60-minute 37°C incubation with 6 of 6 cells and the autocontrol. In a saline tube IAT-AHG, 1–2+ reactivity was observed with all cells and the autocontrol (2+). An acid eluate was positive with group O, ficin-treated RBCs by PEG tube method. The patient's plasma was adsorbed three times with ZZAP-treated autologous RBCs and tested against a standard panel at 37°C (60 minutes, saline tube) and IAT-AHG. Anti-Jk[b] was identified in the IAT-AHG; no other alloantibody specificities were noted. The patient's RBCs were typed as Jk(a+b–).

A review of the patient's transfusion records indicated seven previous RBC transfusions during the course of chemotherapy at another hospital, with the last transfusion 8 months earlier. According to this hospital's records, his antibody detection test

Table 3. Laboratory findings in patients with therapy-related anemia due to checkpoint inhibitors

Reference	Hematologic diagnosis	DAT	Hemolysis	Peripheral blood	Bone marrow
16	WAIHA	IgG+	Yes	Anemia	Erythroid hypoplasia
	Pure RBC aplasia	C3–		Reticulocytopenia	C3+ T-cell lymphoid infiltrate
14	WAIHA	Positive	Yes	Reticulocytosis	Not done
				Spherocytes	
15	WAIHA	IgG+	Yes	Reticulocytosis	Not done
				Agglutination	
				Spherocytes	
14	Anemia	NA	No	Normal RBC	Lymphocytic infiltrate
12	Pancytopenia	Negative	No	Anemia	Hyperplastic marrow
				Thrombocytopenia	Erythroid hyperplasia
				Granulocytopenia	Myeloid hypoplasia
				Hyperlymphocytosis • CD4+CD8+ T-cells • NK-cells	Atypical lymphocytosis Large granular lymphocytes
13	Pure RBC aplasia	Positive	No	Anemia*	Normocellular marrow
				Reticulocytopenia	Erythroid hypoplasia
					Lymphoid aggregates
Patient 1	Warm and cold RBC	IgG+	No	Anemia	Not done
	autoantibodies	C3+		Mild lymphopenia	
Patient 2	Warm RBC	IgG+	No	Anemia	Not done
	autoantibodies	C3+		Mild lymphopenia	

*Anemia due to ongoing blood loss.
DAT = direct antiglobulin test; WAIHA = warm autoimmune hemolytic anemia; RBC = red blood cell; NA = not available.

was negative on four out of four occasions. The patient's other medications included iron, folate, tamsulosin, ibuprofen, and acetaminophen/hydrocodone.

The patient has now received 14 cycles of nivolumab with no evidence of disease progression. His course was complicated by painful arthritis of his hands early during therapy, which has improved with daily ibuprofen and time. The patient did not receive steroids or other immunosuppression medications. His blood counts remain stable (Hb 8.7–9.5 g/dL) with a mild absolute lymphopenia (800–1000/µL) and no evidence of hemolysis. There have been no repeat immunohematology studies in the last 6 months.

Discussion

Ipilimumab and other checkpoint inhibitors enhance cellular and humeral immunity to cancer at the expense of immune tolerance to self-antigens.[1,5] As a consequence, up to 60–70 percent of patients receiving ipilimumab experience at least one irAE during the course of induction therapy.[1,3] Although most irAEs are mild, approximately 18 percent of patients have severe grade 3 or 4 toxicity, with rare fatalities (0.6%).[1,3,5] Patients with prior antibody-mediated immune disorders have had recurrence after starting therapy.[12] Clinicians are cautioned to monitor patients closely for irAEs with a low index of suspicion to initiate treatment. Steroids remain as the first-line treatment for most irAEs.[1] Symptoms generally resolve within 4–8 weeks of discontinuing treatment.[1]

Although considered a rare irAE (<1%), immune-mediated cytopenias have now been reported in several patients (Table 2).[3,4,12–16] Anemia is the most common cytopenia and may be accompanied by thrombocytopenia and/or neutropenia. Anemia may be either hemolytic or hypoproliferative; one patient presented with severe anemia due to both WAIHA and pure RBC aplasia.[16] Anemia is often accompanied or preceded by other irAEs of the skin, gastrointestinal, and endocrine systems. Like other irAEs, immune cytopenias tend to occur during induction therapy (weeks 1–12),[4,12,14,15] although there are examples of patients developing anemia many months after starting immunotherapy.[13,14] Interestingly, some patients have tolerated ipilimumab with no irAEs, only to develop a hemolytic anemia following subsequent treatment with an anti-PD-1 inhibitor.[15,16] Anemia can be steroid-resistant and subject to relapse.

In laboratory testing, over 50 percent of patients with irAE anemia will have evidence of a positive DAT, usually with IgG (Table 3). In three out of five patients with a positive DAT, hemolysis was present.[14–16] In four of four patients who underwent a bone marrow biopsy, a lymphocytic infiltrate was observed.[12–14,16] In one case, an atypical lymphoid infiltrate was accompanied by an absolute and relative increase in circulating lymphocytes composed of NK cells and immature CD4+CD8+ lymphocytes.[12]

Our two patients showed a typical presentation, with the development of new erythroid autoantibodies during induction therapy. Like many patients (Table 2), our patients also developed other irAEs (pruritic rash, arthritis). One unusual finding in our two patients was a positive DAT by both IgG and C3, and one patient demonstrating cold and warm autoantibodies in her plasma. Neither patient had laboratory evidence of hemolysis. Patient 1 did have significant anemia early in her course that was attributed to chronic blood loss, although her Hb stabilized after transfusion and additional treatment.

In addition to these two patients, we may have encountered a third case of drug-related autoantibodies in a 72-year-old woman with metastatic adrenal carcinoma. The patient presented with a positive DAT (IgG 3+, C3 3+) with a pan-reactive warm autoantibody (IAT 2+) after participating in a PD-L1 trial (atezolizumab) at another institution. Interestingly, she was withdrawn from the study trial because of an irAE, with markedly elevated liver enzymes.

The possible mechanisms underlying ipilimumab-associated immune cytopenias may be inferred from studies of other common ipilimumab-associated irAEs. Biopsies of affected skin, gastrointestinal tissue, and liver typically show a lymphocytic infiltrate, sometimes accompanied by other inflammatory cells.[1,3] This result is consistent with increases in CXCL2 and IL8 receptor ligands, which promote leukocyte trafficking.[4] There is also evidence of upregulation of perforin, CD8, Th1 cells, and IFN-γ inducible genes and downregulation of IL-10, an antiinflammatory cytokine.[1,4,20] In peripheral blood, there is a 50 percent decrease in CD4+ Treg cells and an increase in CD4+ effector cells and Th17 cells.[4,20] The loss of Treg cells, in particular, may tip the balance toward an immune cytopenia and WAIHA.[21] CD4+CD25+ Treg cells are believed to play a critical role in the pathogenesis of RBC autoantibodies and WAIHA.[22–24]

In summary, we present two cases of RBC autoantibodies in patients receiving immunotherapy with checkpoint inhibitors. These two patients were identified because of chronic anemia (blood loss, recent chemotherapy) requiring a routine type and screen and blood transfusion. Although the timing of autoantibody development is consistent and highly suspicious for a drug-induced irAE due to checkpoint

inhibitor therapy, we cannot definitely prove causality given the small number of patients and the retrospective nature of this report. As more oncology patients are treated with checkpoint inhibitors, it is probable that additional cases will be encountered. The true incidence and risk of autoantibody formation with these drugs, however, will require larger studies and prospective monitoring of patients over the course of therapy. Immunohematology reference laboratories should be aware of the possibility of cytopenias and autoantibodies in cancer and bone marrow transplant patients receiving these medications.

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