Severe treatment-refractory T-cell-mediated immune skin toxicities observed with obinutuzumab/rituximab-atezo-pola in two patients with follicular lymphoma

The immune cell microenvironment strongly influences outcome in follicular lymphoma (FL). Checkpoint inhibitors (CPI) and other cancer immunotherapy agents in combination with standard-of-care anti-CD20 antibodies (obinutuzumab [G] or rituximab [R]) are currently being evaluated in FL; a phase 1/2 trial in relapsed/refractory (R/R) FL (BO29561; NCT02729896), is evaluating G or R combined with the anti-programmed death-ligand 1 (PD-L1) antibody, atezolizumab, and the anti-CD79b antibody–drug conjugate, polatuzumab vedotin (G/R-atezo-pola). An ongoing phase 1b study has demonstrated the activity and low rate of immune-mediated toxicities with G-atezo in R/R non-Hodgkin lymphoma.[1] Furthermore, two phase 1b/2 studies in R/R FL and diffuse large B-cell lymphoma (DLBCL) have reported acceptable safety profiles with G-pola.[2,3] CPI are associated with specific immune-related toxicities that mainly involve the gut, skin, endocrine glands, kidneys, liver and lungs.[4] Guidelines from the American Society of Clinical Oncology suggest that these events are manageable with corticosteroids.

Among 13 R/R FL and 21 R/R DLBCL patients treated with G-atezo-pola and R-atezo-pola, respectively, in the BO29561 trial, we present two case reports of R/R FL patients who died while experiencing drug-related toxicity. Patients experienced a constellation of immune toxi-

Table 1. Summary of clinical presentation and management of events.

Patient 1: Obinutuzumab 1,000 mg (D1, D8, D15 of C1; D1 of C2-6), atezolizumab 1,200 mg (D1 of C2-6) and polatuzumab 1.8 mg/kg (D1 of C1-6); 1 cycle = 21 days							
Study day onset	**74-79**	**84-100**	**106-113†**	**118-152**	**157-179**	**181-239**	**309-323**
Immune-related reactions							
Dermatitis	Grade I (D74) including facial edema	Facial edema resolved	Grade III (D106*)	Grade II (D121)	Grade III (D161)	Grade II (D209)	
Stomatitis	Grade I (D74) Grade II (D77) Grade II (D79)	Grade II	Grade III (D111)	Grade I (D121)	Grade II (D161) Grade II (D157)		
Keratoconjunctivitis sicca	Grade 2 (D74)	Grade I	Grade II (D108)		Grade I (D168)	Grade II (D197)	
Systemic and non-systemic immunosuppressive therapies							
Prednisolone	10 mg/d (D77) to 100 mg/d IV (D78)	100 mg/d oral (D84); tapered down to 20 mg/d (D86-100)	*Single dose 180 mg IV (D106); 100 mg/d (D107)	D118; tapered down from 90 to 40 mg/d (D118-152)	30 mg/d (D159); 50 mg/d (D161); 100 mg/d (D164); then tapered down from 80 to 70 mg/d (D173-179)	Tapered down from 60 to 5 mg/d (D186-239) →	
Infliximab			425 mg IV q2w (D112) →				
Tacrolimus					1 mg/d 4-6 µg/L PO (D164) →		
Anakinra						100 mg/d SC (D203)	
Complications from long-term immunosuppressive treatment							
Erysipelas				Grade II (D128); resolved ~3 months later			
Oral fungal infection				Grade II (D128); resolved D148	Grade I (D157)		
Bronchopulmonary aspergillosis					Grade II (D160); resolved ~4 months later		
Viral infection					Grade I (D157); resolved ~2 months later		
Persistent Gram-negative bacteremia						Grade II (D181 and D200); resolved ~2 weeks later	

Patient 2: Obinutuzumab 1,000 mg (D1, D8, D15 of C1; D1 of C2-6), atezolizumab 1,200 mg (D1 of C2-6) and polatuzumab 1.8 mg/kg (D1 of C1-6); 1 cycle = 21 days							
Study day onset	**41-46**	**47-48**	**50-61**	**68-70**	**76-78**	**82-85**	**90-95**
Immune-related reactions							
Pneumonitis	Grade III (D41)	Grade II (D47)	Grade I (D51)			Grade II (D82) Grade II I (D85)	
Erythema			Grade II (D50)		Grade I (D77)		
Stomatitis			Grade II (D50) Grade III (D57)	Grade II (D68)	Grade I (D77)		
Systemic and non-systemic immunosuppressive therapies							
Prednisolone	500 mg/d IV (D41) 500 mg IV bid (D42-46)	Tapered down from 500 mg IV bid to 20 mg/d PO (D47-48)	500 mg/d IV (D50) Tapered down from 500 to 80 mg/d IV (D51-58) →		Tapered down from 80 to 60 mg/d IV (D76)	100 mg/d IV (D82); 500 mg/d IV (D85)	Tapered down from 500 to 200 mg/d IV (D90-93)
Tocilizumab	3000 mg IV (D44)					300 mg IV (D85)	
Budesonide		250 mg/mL inhalation, 1 mL/d (D48)	250 mg/mL inhalation, 1 mL bid (D61)	250 mg/mL inhalation, 1 mL/d (D71) →		250 mg/mL inhalation, 1 mL bid (D86) →	
Tacrolimus			2 mg (5 ng/mL) PO bid (D56) then tapered down to 1 mg PO bid (D61) →		1 mg (5 ng/mL) qd (D76) →		
Complications from long-term immunosuppressive treatment							
Bronchopulmonary aspergillosis				Grade III (D68)			Grade IV (D93)

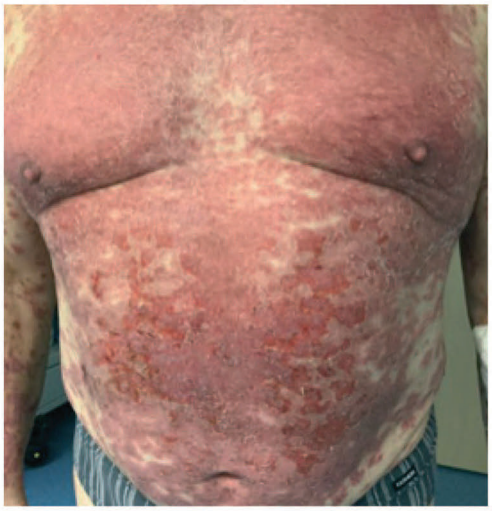
Black arrows represent the duration of the administered immunosuppressive treatment. Bid: twice daily; C: cycle; D: study day; IV: intravenously; PO: orally; q2w: every 2 weeks; qd: once daily; SC: subcutaneously. Histopathological features on skin biopsy include: *(D106 worsening dermatitis) direct immunofluorescence showed 90% of epidermis detached (limited accessibility of the basement membrane zone and epidermis) and deposition of the C3 on cytoid bodies; †(D113) profound epidermis necrosis, bullous spaces in basal layer, PD-L1 overexpression, T-cell infiltration ($CD8^+$ predominating over $CD4^+$), and the absence of B cells.

cities (concomitant severe dermatitis, stomatitis, and ocular) that were refractory to standard immunosuppressive treatment with systemic corticosteroids, and suggestive of Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) or resemble the features of chronic graft-*versus*-host-disease (GvH) as summarized in Table 1.

Patient 1, a 68-year-old male with stage IV R/R FL and a prior history of lichen simplex chronicus (resolved in 2014), previously received treatment with R-bendamustine (in 2013; achieved a partial response), followed by rituximab maintenance (2013-2015), R-bendamustine and venetoclax (in 2016; achieved a complete response). In 2017, he started treatment with G-atezo-pola and achieved a durable complete response post-induction.

He initially presented with grade I dermatitis and stomatitis, and grade II keratoconjunctivitis sicca on day 74 (induction cycle 3), around 10 days after the third dose of atezo, fourth dose of pola, and sixth dose of G. Initial symptoms improved following systemic prednisolone. Following steroid tapering, the patient was hospitalized due to rebound toxicities (Figure 1A-B). Histopathological features included full-thickness epidermal necrosis and subepidermal blistering with an epidermotropic lymphocytic infiltrate – features suggestive of GVH-like disease or toxic epidermal necrosis (Table 1; Figure 1C). Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), to detect auristatin deposits, the cytotoxic component of polatuzumab vedotin, was inconclusive. Antinuclear antibody tests and rheumatoid factors were negative. The high-grade immune toxicities had a fast onset and rapid progression, especially the skin reactions. The rebound toxicities were refractory to systemic corti-

A



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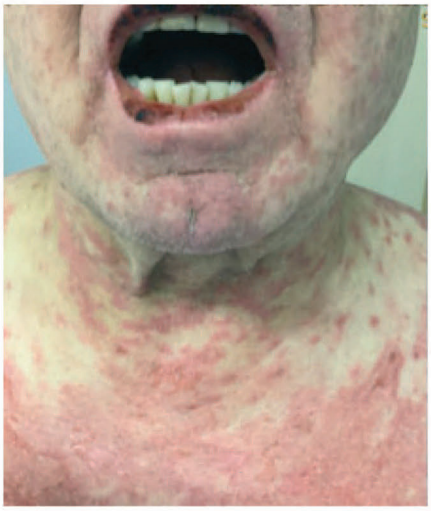

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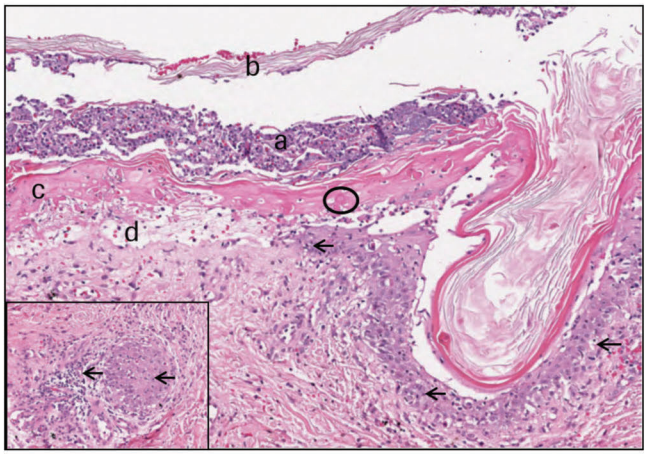
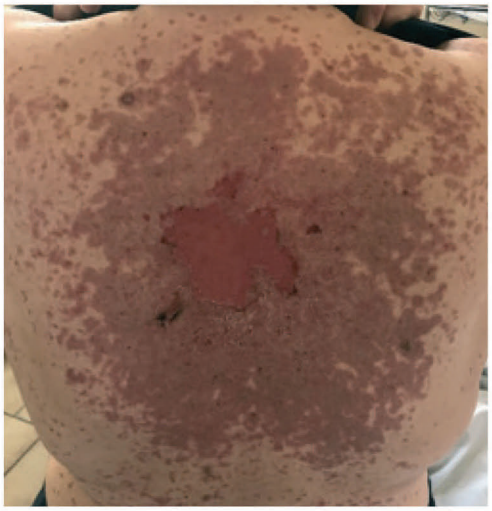


Figure 1. Clinical and histopathological features of the observed immune skin toxicities. (A) High-grade dermatitis in patient 1 with extensive skin abrasions, redness and skin scales, dry skin, and itching; (B) high-grade stomatitis in patient 1; and (C) histopathological diagnostic features in skin biopsy, in patient 1 following presentation of rebound immune-mediated toxicities after steroid tapering: (a) subcorneal pustules with bacterial colonies; (b) basket-weave orthokeratosis; (c) full-thickness epidermal necrosis with cytoid bodies (circle); (d) subepidermal blistering and epidermotropic lymphocytic infiltrate (arrows) involving the hair follicle (inset). Immunohistochemistry (not shown) in patient 1 revealed primarily $CD8^+$ T cells in the lymphocytic infiltrate. (D) Moderate-grade erythematous lesions in patient 2, with merging red erythematous patches without blisters or erosions; (E) moderate-grade stomatitis in patient 2. Immunohistochemistry (not shown) in patient 2 revealed lymphocytic infiltration at the dermis (mostly around the vessels and skin appendages) including neutrophils with disintegration features.

D



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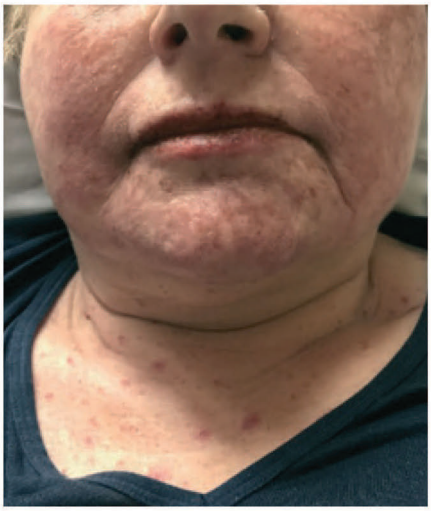

costeroids and difficult to manage (Table 1), requiring immunosuppressive combination treatment, including ciclosporin, infliximab, tacrolimus, and anakinra. The events persisted and slowly evolved to a less reactive, chronic, non-inflammatory state (grade II). He also experienced several immunosuppression-related opportunistic infections (Table 1). Upon stabilization of the grade II events, he was started on rehabilitation. Despite controlling the events with triple immunosuppressive therapy, he died eight months after first onset. No autopsy was performed and the primary cause of death not established.

Patient 2, a 59-year-old female with stage III R/R FL with no prior history of autoimmune reactions, previously received treatment with rituximab + CHOP (in 2015; achieved a complete response), and bendamustine (in 2017; progressive disease). She received treatment with G-atezo-pola, and achieved a partial response at mid-induction.

She presented with grade III pneumonitis and grade II conjunctivitis on day 41 (induction cycle 2), around 20 days after the first dose of atezo, second dose of pola and fourth dose of G (Table 1). Respiratory symptoms improved following high-dose systemic corticosteroid treatment and tocilizumab. Following rapid tapering, she presented with newly onset grade II erythema and grade II stomatitis (Figure 1D-E), in addition to persistent pneumonitis and conjunctivitis. Symptoms improved following treatment with high-dose steroids and tacrolimus. However, she subsequently experienced transaminitis, pulmonary embolism as well as bronchopulmonary aspergillosis and cytomegalovirus infections. Most likely, the intensive immunosuppressive therapy including high dose steroids contributed to these opportunistic infections, despite monitoring of aspergillus antigen in the peripheral blood, and weekly cytomegalovirus DNA monitoring. Approximately four weeks after the first onset of bronchopulmonary aspergillosis, she died. An autopsy revealed bronchopulmonary aspergillosis as the cause of death, with aspergillosis obstruction in the vessels of major organs. At the time of death, skin and ocular lesions, and stomatitis, were resolving. However, pneumonitis was persisting and there was an unconfirmed clinical suspicion of Guillain–Barré syndrome.

The cutaneous, oral and ocular adverse events experienced by both patients are known class-risks for anti-PD-L1/programmed cell death protein-1 (PD-1) inhibitors, albeit at notably lower incidences and with a more benign clinical course.[4,5] These events were considered related to atezo by treating physicians, leading to study treatment discontinuation soon after the first onset of events. The reported cases resemble an autoimmune disease and are consistent with T-cell-driven ($CD8^+$) immune-mediated toxicity.[6,7] The authors hypothesize that the incidence and severity of these events, known to be associated with CPI, may be exacerbated in the context of a profound dysregulation of the immune system: obinutuzumab- and polatuzumab vedotin-mediated B-cell suppression, and in particular, regulatory B-cell depletion.[6,7] Both cases share clinical and histological features with a spectrum of clinical entities that span between SJS/TEN and the features of chronic GVHD disease. Clinical risk factor analysis, including review of prior and concomitant therapies, relevant medical history, and pre-treatment T-cell counts, did not suggest any baseline characteristics that could help identify patients at high risk for developing these toxicities.

The results of the skin biopsies suggest several mechanisms for the pathophysiology of the immune-mediated toxicities, and dermatitis in particular.[8] $CD8^+$ T-cell infiltration into the epidermis junction is suggested as the primary mechanism of epidermal cytotoxicity. The increased PD-L1 expression is consistent with a mechanism of preservation of epidermal integrity during inflammatory skin reactions.[9] $CD8^+$ T-cell hyperactivation resulted in PD-L1 overexpression in the surviving epidermis, consistent with the tolerogenic role of the PD-L1/PD-1 pathway.[10,11] Of note, both patients had received prior treatment with bendamustine, which has been reported to induce regulatory T-cell depletion,[12] potentially contributing to the immune dysregulation in these patients. There is insufficient evidence to support an auristatin-derived direct toxicity, as MALDI-MS performed on the skin biopsy of patient 1 was inconclusive.

While mucosal, ocular, and cutaneous toxicities have been observed with anti-PD-L1/PD-1 therapies,[4,5] the constellation of concomitant toxicities, as well as the unfavorable treatment-refractory severe clinical course of these cases with G-atezo-pola, are not consistent with the safety profile of the individual study drugs, or the double combinations of G-atezo or G-pola.[1,2,13-15] Rather, we propose that the observed toxicities may have been exacerbated when administered concomitantly in this triplet combination. Further, in light of the treatment-refractory course of the clinical constellation, and the necessity of prolonged immunosuppression in these cases, prophylaxis may be needed for opportunistic infections and other complications of immunosuppression. Of note, similar serious immune-mediated adverse events have not been described in studies evaluating the combination of PD-L1/PD-1 inhibitors and B-cell depleting therapy with either rituximab/obinutuzumab[16-18] or CD19 targeted CAR-T therapy[19] suggesting that these combinations may have an acceptable safety profile. Interestingly, Das *et al.* evaluated whether changes in circulating B cells in combined checkpoint blockade-treated patients correlated with an increased risk or severity of immune-related adverse events.[20] The authors found that patients with a ≥30% reduction in baseline levels of total circulating B cells were significantly more likely to develop high-grade immune related adverse events than those without a reduction in circulating B cells.[20] In addition, early changes in circulating B cells after only one round of combination checkpoint blockade correlated with a median time of three weeks to immune-related adverse event onset.[20] These observations may support the effect of B-cell depletion on the exacerbation of immune-mediated adverse events in patients exposed to single-agent checkpoint blockade.

In conclusion, these two cases feature a severe and difficult-to-treat T-cell-driven immune-mediated constellation of events, which contributed to the death of two patients. Both cases were complicated by opportunistic infections, likely resulting from the intense immunosuppressive therapy required to manage the events. The observed constellation of toxicities appear specific to the combination of G-atezo-pola. No such toxicities were observed in the R-atezo-pola cohort; however, given the similar class combination and mechanism of action, an association cannot be excluded. Overall, based on the sponsor's assessment of the two cases, the benefit/risk profile for G-atezo-pola in patients with R/R FL and for R-atezo-pola in patients with R/R DLBCL is unfavorable. Enrollment into the BO29561 trial was stopped and atezo was discontinued in all ongoing patients. No further development of G/R-atezo-pola combinations is planned.

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