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Retrospective analysis of a single-center clinical experience toward development of curative treatment of 123 pemphigus patients with a long-term follow-up: efficacy and safety of the multidrug protocol combining intravenous immunoglobulin with the cytotoxic immunosuppressor and mitochondrion-protecting drugs

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Abstract

Background Pemphigus vulgaris (PV) is a life-long IgG autoantibody-mediated blistering disease affecting the mucosal surfaces lined by the stratified epithelium (oral, nasal, genital) and sometimes also the skin. While corticosteroid treatment is life saving, the high dose and prolonged courses required for disease control are associated with significant adverse effects, including death. Although introduction of rituximab (RTX) provided for a favorable outcome, the high relapse rate, that is, up to 80%, precludes successful use of RTX as a monotherapy. Intravenous immunoglobulin (IVIg) is being increasingly utilized as off-label therapy for a variety of autoimmune and inflammatory diseases, including PV and pemphigus foliaceus (PF).

Aims The goal of pemphigus research is to develop an effective treatment modality that would allow patients to achieve and maintain a stable clinical remission without the need for additional treatments, or cure.

Materials and Methods This article summarizes clinical outcome of 123 pemphigus patients treated with a combination of IVIg, an immunosuppressive cytotoxic drug (ICD) and mitochondrion-protecting drugs in the Blistering Disease Clinic at the University of California, Irvine from 2007 to 2017.

Results The mean time to disease control was 0.2 months and time to complete remission – 1.7 months. Duration of complete remission on drugs until relapse or end of treatment was 19.3 months. The mean duration of complete remission off drugs until relapse was 15.8 months. That until end of follow up was 48.4 months, with a minimum of 14 and a maximum of 91 months. The overall complete remission rate off all drugs was 100%, with 12% overall relapse rate. Most relapses, 8.1 vs. 3.3%, occurred during the time of treatment, compared to posttreatment. No patients had more than a single relapse. The duration of the posttreatment follow-up ranged from 9 to 97 months with a mean of 64.8 months, or 5.4 years. The total number of IVIg cycles ranged from 26 in patients without a relapse to 37 in patients with a relapse. The clinical outcome in patients that received IVIg with RTX or another ICD were found to be very similar.

Discussion Thus, the multidrug IVIg regimen allowed to achieve three principal treatment objectives: (i) rapid control of pemphigus symptoms; (ii) stable disease remission; and (iii) overall safety of treatment.

Conclusions While the individualized therapeutic approaches to eradicate the autoreactive B cell clones causing disease in each particular PV or PF patient are being developed, all pemphigus patients can benefit from the treatment protocol described in this study.

Introduction

Pemphigus vulgaris (PV) is a life-long IgG autoantibody-mediated blistering disease affecting the mucosal surfaces lined by the stratified epithelium (oral, nasal, and genital) and sometime also the skin. While the corticosteroid treatment is life saving, the high-dose and prolonged courses required for disease control are associated with significant adverse effects, including death.[1,2] The hazardous side effects of conventional immunosuppressive therapy with high-dose systemic corticosteroids requiring prolonged and frequent hospitalizations make PV therapy very expensive[3] and necessitate meticulous care of a wide array of comorbid health conditions.[4] A systematic review and meta-analysis of randomized controlled trials evaluating the available evidence regarding efficacy and safety of interventions for PV concluded that despite their widespread use, it is not known if steroid-sparing agents are beneficial.[5]

Although introduction of rituximab (RTX) provided for a favorable outcome, the high relapse rate, that is, up to 80%,[6] precludes successful use of RTX as a monotherapy. Intravenous immunoglobulin (IVIg) is being increasingly utilized as off-label therapy for a variety of autoimmune and inflammatory diseases, including PV and pemphigus foliaceus (PF) (reviewed in[7]). In contrast to RTX and conventional immunosuppressive agents (ISA), IVIg does not cause immunosuppression that endangers patients with infectious complications. It has been demonstrated that coadministration of an immunosuppressive cytotoxic drug (ICD) improves the ability of IVIg to lower serum levels of pathogenic autoantibodies in pemphigus.[8,9] However, to definitively conclude about efficacy and safety of treatment protocols incorporating IVIg, the extended follow-up observations of large cohorts of patients is required.

This article summarizes clinical outcome of 117 PV and six PF patients treated with a combination of IVIg, an ICD, and mitochondrion-protecting drugs in the Blistering Disease Clinic at the University of California, Irvine from 2007 to 2017. To the best of my knowledge, this is the largest cohort of pemphigus patients reported to be treated with IVIg in a single academic center and followed up for a sufficiently long period of time to justify meaningful conclusions. The results have demonstrated a success rate of 100%, suggesting that permanent remission off drugs (or cure) may be possible.

Patient characteristics

Medical records of a total of 117 PV and six PF patients (55 males and 68 females) aged 6–89 were analyzed retrospectively. All patients had active disease on the first visit, and all had been treated with prednisone and/or ISA in the past. Prior to initiation of treatment with the multidrug protocol, the mean duration of the disease was 2.7 years, ranging from 2 months to 16 years. Among these 123 patients, 32 PV and four PF patients had refractory disease, 79 PV and two PF patients had relapse, and six PV patients were previously untreated. All previously treated patients received prednisone together with conventional ISAs, that is, mycophenolate mofetil (84 PV and four PF patients), azathioprine (58 PV and two PF patients), methotrexate (four PV patients), and cyclophosphamide (three PV patients). RTX was used to treat eight PV patients with refractory disease and 31 PV and two PF relapsed patients. The diagnosis of PV and PF was made based on results of comprehensive clinical and histological examinations, and immunological studies that included direct immunofluorescence of oral and/or skin biopsies, indirect immunofluorescence (IIF) of the patients' sera on various epithelial substrates, and ELISA for anti-desmoglein (Dsg) 1 and anti-Dsg 3 antibodies. The mucosal type of PV was diagnosed in 23 and the mucocutaneous type in 94 PV patients. The severity of disease was determined using the original "Pemphigus Disease Area and Activity Index", or PDAAI (Fig. 1), modified from the existing measurement instrument pemphigus disease area index, or PDAI.[10] To optimize the scoring system for continuous monitoring of treatment efficacy, PDAAI excludes secondary skin changes, such as hyperpigmentation, to avoid overrating the disease damage, as patches of postinflammatory hyperpigmentation may be present for a very long time, especially darker-skinned individuals.

Outcome measures

In the past, evaluations of therapeutic interventions in pemphigus were complicated by lack of generally accepted definitions and measurements for the clinical status of PV and PF patients. Fortunately, this problem has been recently resolved. The endpoints in the present study were slightly modified from the international consensus outcome measures for pemphigus,[11] as follows: time to disease control (TDC) – lack of new lesions for a minimum of 2 weeks, and negative Nikolsky sign;[12] time to complete remission (TCR) – complete healing of erosions (with or without secondary skin changes) in the absence of new lesions and with negative Nikolsky sign; duration of complete remission on drugs (DCRon) until a relapse or end of therapy; duration of complete remission off drugs (DCRoff) until relapse or end of follow-up, as well as overall complete remission rate off all drugs, and rate of relapse (overall, and on and off drugs). The relapse was defined as any increase from the previous PDAAI value in a particular patient lasting for at least 2 weeks. During the treatment phase, all patients had follow-up visits at 4–6 week intervals on average and were required to come to clinic immediately upon the onset of new symptoms suggesting a relapse. At each visit, a complete physical examination was made, and routine laboratory tests were performed. The patients who voluntarily altered the treatment protocol were excluded from the analysis. The duration of the posttreatment follow-up ranged from 9 to 97 months with a mean of 64.8 months, or 5.4 years.

Pemphigus Disease Area and Activity Index (PDAAI)

Skin	Blister or erosion or new erythema	Weighting factor for activity (measuring quality of lesions as a multiplier of value for)
	0 absent **1** 1—3 lesions, up to one >2 cm in any diameter, none >6 cm **2** 2—3 lesions, at least two >2 cm in any diameter, none >6 cm **3** >3 lesions, none >6 cm in diameter **5** >3 lesions, and/or at least one >6 cm **10** >3 lesions, and/or at least one >16 cm in diameter or entire area	**x 1.5** positive Nikolsky sign **x 1** presence of blister fluid and/or exudate on the erosion surface **x 0.5** dry (re-epithelializing) erosion
Ears		
Nose		
Rest of the face		
Neck		
Chest		
Abdomen		
Back, buttocks		
Arms		
Hands		
Legs		
Feet		
Genitals		
Total skin		**/180**

Scalp	Blister or erosion or new erythema	Weighting factor for activity (measuring quality of lesions as a multiplier of value for)
	0 absent **1** in one quadrant **2** two quadrants **3** three quadrants **4** affects whole skull **10** at least one lesion >6 cm in diameter	**x 1.5** positive Nikolsky sign **x 1** presence of blister fluid and/or exudate on the erosion surface **x 0.5** dry (re-epithelializing) erosion
Total scalp		**/15**

Mucosa	Blister or erosion or new erythema	Weighting factor for discomfort (measuring quality of lesions as a multiplier of value for)
	0 absent **1** 1 lesion **2** 2-3 lesions **5** >3 lesions or two lesions >2 cm **10** entire area	**x 1.5** always painful/bleeding **x 1** some time painful/bleeding **x 0.5** never painful/bleeding
Eyes		
Nose		
Buccal mucosa		
Hard palate		
Soft palate		
Upper gingiva		
Lower gingiva		
Tongue		
Floor of mouth		
Labial mucosa		
Posterior pharynx		
Anogenital		
Total mucosa		**/180**

Grand total

Figure 1 A spreadsheet for measuring Pemphigus Disease Area and Activity Index (PDAAI). Modified from.[71]

Treatment protocol

All treatment modalities included in the multidrug protocol described below were initiated simultaneously. All patients were treated with oral prednisone or methylprednisolone starting at approximately 1 mg/kg/day dose, and the mitochondrion-protecting drugs minocycline (or doxycycline) at 200 mg/day and niacinamide (nicotinamide) at 1.5 g/day. In case of disease progression, the dose of prednisone was increased by approximately 20% in a 2-week interval until disease control was achieved. In patients who did not respond to approximately 1.5 mg/kg per day, prednisone was changed to the equivalent in activity dose of methylprednisolone, taking into consideration that the activity of 5 mg of prednisone is equivalent to that of 4 mg of methylprednisolone. After disease control was achieved, the therapeutic dose of prednisone/methylprednisolone remained fixed until complete remission of disease, at which point the daily dosage was reduced by 15–20% every 15–20 days down to zero, as originally described by Dr. Lever.[13,14] IVIg, 2 g/kg/month, was given on 4–5 consecutive days as a slow, approximately 4-hour infusion, hereinafter referred to as "IVIg cycle." Prior to each infusion, the patients were premedicated with 50 mg diphenhydramine and 500 mg acetaminophen. To prevent a rebound effect from compensatory overproduction of pemphigus antibodies by the autoantibody-producing cells stimulated via a negative feedback due to rapid clearance of pathogenic IgGs,[15] IVIg was combined with the ICD mycophenolate mofetil 2 g/day, azathioprine 200 mg/day, or cyclophosphamide 150 mg/day, chosen in the above order depending on the patient's insurance allowance as well as counter-indications and adverse reactions (Fig. 2a). The choice of ICD depended on the baseline blood cell count and the biochemistry indices reflecting liver and kidney functions. If allowable by insurance, mycophenolate mofetil was used as the first choice. If not, the patients were treated with azathioprine or cyclophosphamide. The patients who could not tolerate ICDs were switched to RTX, which was administered using the modified IVIg+RTX protocol published by Ahmed *et al.*,[16] according to which RTX was given at 375 mg/m^2 body surface area once per week for 3 weeks during the first and the second months followed by four monthly infusions (10 infusions total; Fig. 2b). Pemphigus patients with disease relapse were treated exactly the same way as new patients, that is, the current dose of prednisone/methylprednisolone was increased by 50–100%[13,14] until disease control, and then tapered off per Dr. Lever's protocol. After prednisone/methylprednisolone was tapered off, all other medications were continued without a change for 6 months. If no relapse was observed during that time, the frequency of IVIg was decreased to every other month for three cycles and then to every three months for two cycles (Fig. 2a). Thus, in the absence of relapses, the post-steroid treatment continued for 18 months. All patients also received 1 g of calcium supplement and two age/gender-specific multivitamin tablets per day, as well as medications required to control coexisting medical conditions and complications, if any. The treatment ended, that is, all oral medicines were discontinued, two weeks after the last IVIg cycle. The patients were counseled about the need to avoid factors known to precipitate pemphigus onset and exacerbations, such as emotional stress,[17–19] overheating,[20,21] ultraviolet radiation[22,23] as well as the drugs angiotensin-converting enzyme inhibitors,[24,25] and penicillin and its derivatives.[26,27]

Results

Clinical results in pemphigus patients treated with IVIg

As shown in Table 1, the retrospective analysis of medical records of 123 pemphigus patients who had completed the

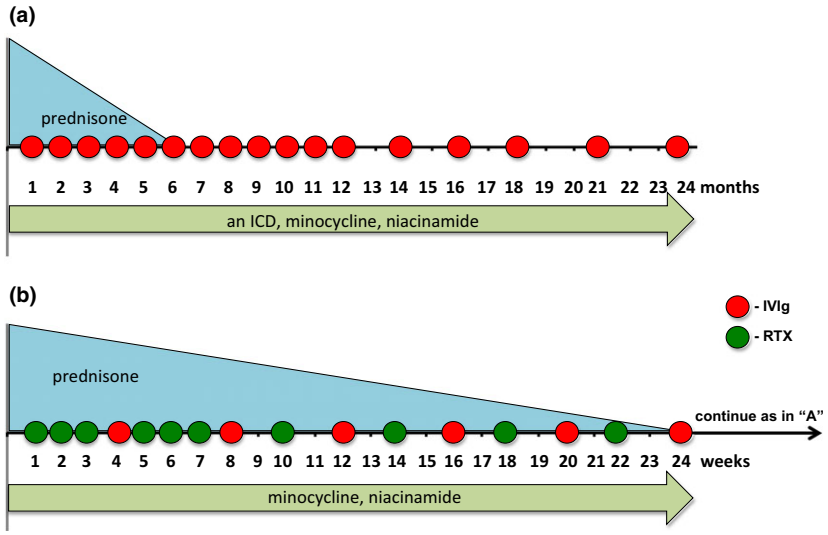

Figure 2 Two scenarios of treatment: without (a) and with (b) RTX.

course of treatment with IVIg demonstrated that the mean TDC was 0.2 months and TCR, 1.7 months. DCRon until relapse or end of treatment was 19.3 months. The mean DCRoff until relapse was 15.8 months. That until end of follow-up was 48.4 months, with a minimum of 9 and a maximum of 97 months. The overall complete remission rate off all drugs was 100% with 12% overall relapse rate. Most relapses, 8.1 vs. 3.3%, occurred during the time of treatment, compared to post-treatment. No patients had more than a single relapse. Current maximum duration of complete clinical remission off all drugs is 91 months or ~7.5 years. The minimal duration of therapy without a relapse was approximately 26 months, but the calculated mean value for all patients, including those who had a relapse, was 29.4 months. Total number of IVIg cycles ranged from 26 (in patients without a relapse) to 37 (in patients with a relapse).

Mycophenolate mofetil was used as the first line ICD in 63 PV and four PF patients and azathioprine in 29 and two PV and PF patients, respectively, and 11 PV patients were initially treated with cyclophosphamide. Transient transaminitis developed in seven patients taking mycophenolate mofetil and two patients taking azathioprine (all had PV). Instead of reducing the standard dose, all patients were switched to cyclophosphamide. There was no evidence that one ICD was tolerated better or associated with a different outcome than others. All patients remained on an ICD throughout the entire duration of and discontinued two weeks after last IVIg cycle together with the mitochondrion-protecting drugs. RTX was included in the IVIg treatment protocol of 23 PV patients who could not tolerate other ICDs due to liver and/or kidney problems or specifically requested RTX. The clinical outcome in patients that received IVIg with RTX or another ICD were similar (Table 1).

Overall, adverse effects of IVIg therapy, that is, those developed during or within 48 hours after IVIg infusion, were observed in 72 patients (58.5%). These complications included mild-to-severe headache (22.1%), nausea/vomiting (14.6%), fever/chills (25.2%), fatigue/lethargy (15.4%), increased or decreased blood pressure (17.9%) and cutaneous symptoms, such as pruritus, erythema, and urticaria (9.8%). These events usually resolved due to slowing the infusion, rate, pausing infusion and/or using symptomatic treatments. Severe intractable headache requiring the need to stop infusion and switch the IVIg batch or brand was observed in seven patients (5.7%), all of whom had a history of migraine. Self-limited anemia, which might be induced by an ICD and/or IVIg (due to natural antibodies to red blood cell antigens), was observed in 12 (9.8%) patients. Rare adverse events requiring hospitalization were observed in five (4.1%) patients. These included pulmonary embolism (1 patient), deep vein thrombosis (1 patient), stroke (1 patient) and hemolytic anemia requiring blood transfusion (2 patients). These complications did not reoccur after IVIg treatment was resumed. All patients with a history of thromboembolic events received anticoagulant therapy.

The *de novo* adverse events developed after initiation of treatment that might not be related to IVIg *per se* included psychological abnormalities, such as agitation, depression and mood swings (9.8%), hypertension (11.4%), diabetes mellitus (8.1%), gastrointestinal distress (4.1%), and transaminitis (6.5%). Most of these complications apparently were caused by corticosteroids and/or ICDs, since they resolved after discontinuation of the offending drug. One PV patient (0.8%) who was treated with azathioprine died from pancytopenia and sepsis. No serious side effects and adverse reactions specific to RTX infusions were observed. In patients who developed transaminitis, a current ICD was replaced with another one and then, if no improvement of liver function tests, with RTX.

The effects of IVIg therapy with RTX or another ICD on circulating pemphigus autoantibody levels

The IVIg treatment with an ICD or RTX produced similar results on the serum levels of pemphigus autoantibodies (Fig. 3). By the time of onset of complete clinical remission (i.e, ~1.7 months after treatment initiation), the number of patients with positive serum autoantibody tests decreased by 20–50%, and by the end of treatment (i.e, ~29.4 months after treatment initiation) by 60–90%. By the end of the first year post-treatment, all patients had negative IIF (Fig. 3). In contrast, approximately 8 and 28% of patients remained positive for anti-Dsg 1 and ant-Dsg 3 antibodies, respectively (Fig. 3).

Discussion

The results of comprehensive treatment of our patients with PV and PF using the multidrug protocol that consisted of the initial loading dose of prednisone and prolonged administration of IVIg together with an ICD and mitochondrion-protecting drugs allowed to achieve three principal treatment objectives: (i) rapid control of pemphigus symptoms; (ii) stable disease remission; and (iii) overall safety of treatment. Although our therapeutic approach did not ultimately resolve the principal problem of pemphigus treatment because of remaining issues with adverse events from systemic corticosteroids and ICDs as well as a relatively long duration of treatment, it allowed to achieve a prolonged, possibly lifetime clinical remission in a vast majority of pemphigus patients.

Administration of our multidrug treatment protocol allowed to rapidly achieve and maintain a disease remission in 100% of patients requiring only a limited use of prednisone/methylprednisolone, that is, during approximately 5 months at the beginning of treatment. The relative cumulative dose of systemic corticosteroids can be estimated based on the fact that during the TCR, which lasted 1.7 months on average, the daily dose of prednisone was approximately 1 mg/kg/day and then reduced by 15–20% every 15–20 days down to zero. The last daily dose of corticosteroids before discontinuation was 2.5 mg of prednisone and 2 mg of methylprednisolone. Therefore, a 75 kg

Table 1 Summary of clinical parameters in pemphigus patients treated by different regimens.

	This study					Literature reports				
	While on multidrug IVIg treatment protocol with ICD	**After completion of multidrug IVIg treatment protocol with ICD**	**While on multidrug IVIg treatment protocol with RTX**	**After completion of multidrug IVIg treatment protocol with RTX**	**Average for all patients**	**While on IVIg+RTX treatment protocol**	**After IVIg+RTX treatment protocol**	**While on RTX treatment protocol without IVIg**	**After RTX treatment protocol without IVIg**	**While on treatment protocol without IVIg and RTX**
Time to disease control (TDC): no new lesions, Nikolsky sign becomes negative	0.2 months (6 days)	n/a	0.3 (9 days)	n/a	0.2 months (7 days)	0.7 months (22 days)[a] 1 months[49,50]	n/a	Overall mean: 1.1 months[b] 1.1[60]	n/a	38 days (1.2 months)[28]
Time to complete remission (TCR)	1.8 months (54 days)	n/a	1.6 (48 days)	n/a	1.7 (50 days)	1.7 months (52 days)[a] 2.1 months[49]	n/a	Ranges 2.4-4.2 months depending on number of RTX infusions[51,52] Overall mean: 5.8 months[b]	4.4 months[60]	177 days (5.9 months)[28] 4.3 months[54]
Complete remission duration on drugs until relapse or end of treatment (DCRon)	22.9 months Minimum: 6.2 months Maximum: 26.4 months	n/a	16.2 months Minimum: 9.5 months Maximum: 26.1 months	n/a	19.3 months	79.3 months (after RTX was stopped and IVIg continued for additional 17.6 months)[a]		Ranges 9.8-17.1 months depending on number of RTX infusions[51] Overall 14.5 months[b]	DNA	DNA
Complete remission duration off drugs until relapse or end of follow-up (DCRoff)	n/a	Average until relapse: 16.1 months Average until end of follow-up: 57.4 months Minimum: 14 months Maximum: 91 months	n/a	Average until relapse: 12.6 months Average until end of follow-up: 32.7 months Minimum: 14.3 months Maximum: 52.0 months	Average until relapse: 15.8 months Average until end of follow-up: 48.4 months	n/a	80.3 months (after both RTX and IVIg were discontinued)[a] 120 months[49,50]		Overall mean: 13.1 months[52] 17.5 months[60] 14.5 months[b]	DNA
Overall complete remission rate off all drugs, %	n/a	100	n/a	100	100	100[a]	100[a] 100[49,50]	100%[51] 75.8%[b]	37%[52] 88%[60] 38.7%[b]	44% after 6.4 yrs[58] 50% after 5 yrs[72] 70.6% (“minimal”, i.e, <10 mg/days prednisone or off all drugs[61]
Rate of relapse on drugs, %	8.0	n/a	8.7	n/a	8.1	0[a] 0[49,50]	0[a] 0[49,50]	DNA	n/a	26% with or without drugs – unknown[58]
Rate of relapse off drugs, %	n/a	3.0	n/a	4.3	3.3	0[a] 0[49,50]	0[a] 18[49,50]	n/a	46% – average for different protocols at 15.3 months follow-up[59] 16%[60]	22%[28]

Table 1 Continued

	This study					Literature reports				
	While on multidrug IVIg treatment protocol with ICD	After completion of multidrug IVIg treatment protocol with ICD	While on multidrug IVIg treatment protocol with RTX	After completion of multidrug IVIg treatment protocol with RTX	Average for all patients	While on IVIg+RTX treatment protocol	After IVIg+RTX treatment protocol	While on RTX treatment protocol without IVIg	After RTX treatment protocol without IVIg	While on treatment protocol without IVIg and RTX
Overall rate of relapse, %	8.0	3.0	8.7	4.3	12	0[a]	0[a] 18[49,50]	44.4%.[52] 40.2%[b]	80% after 7 yrs follow-up among those surviving >2 yrs post first RTX treatment[6] Ranges 50-79% depending on number of RTX infusions[51]	31% with or without drugs – unknown[61] 39.8% – "major" and 71.1% – minor relapses[73]
Mean duration of therapy	30.2 months	n/a	28.6 months	n/a	29.4 months	23.7 months[a] 6 months[49,50]	n/a	Depends on protocol[c]	n/a	36 ± 39 months[58]
Average rate of adverse events causing hospitalization	3.0	n/a	8.7	n/a	4.1	0[a] 0[49,50]	n/a	3.3[b]	n/a	11.5%[28]
Mortality rate, %	1.0	n/a	0	n/a	0.8	0[c] 0[49,50]	n/a	0-12.5 – reviewed in[59] 13.6[6]	n/a	4.8[74] 1.4–7 – reviewed in[75] 12[76]

DNA, data not available; ICD, immunosuppressive cytotoxic drug; n/a, not applicable; RTX, rituximab.
[a]First line IVIg+RTX treatment without systemic corticosteroids[50]
[b]Meta analysis[53]
[c]Lymphoma protocol is four weekly RTX infusions of 375 mg/m^2 RTX; rheumatoid arthritis protocol is 500 mg ("low dose") or 1000 mg RTX on days 1 and 15; modified protocols are variations in the dosage and/or the duration of treatment (reviewed in[59,77]).

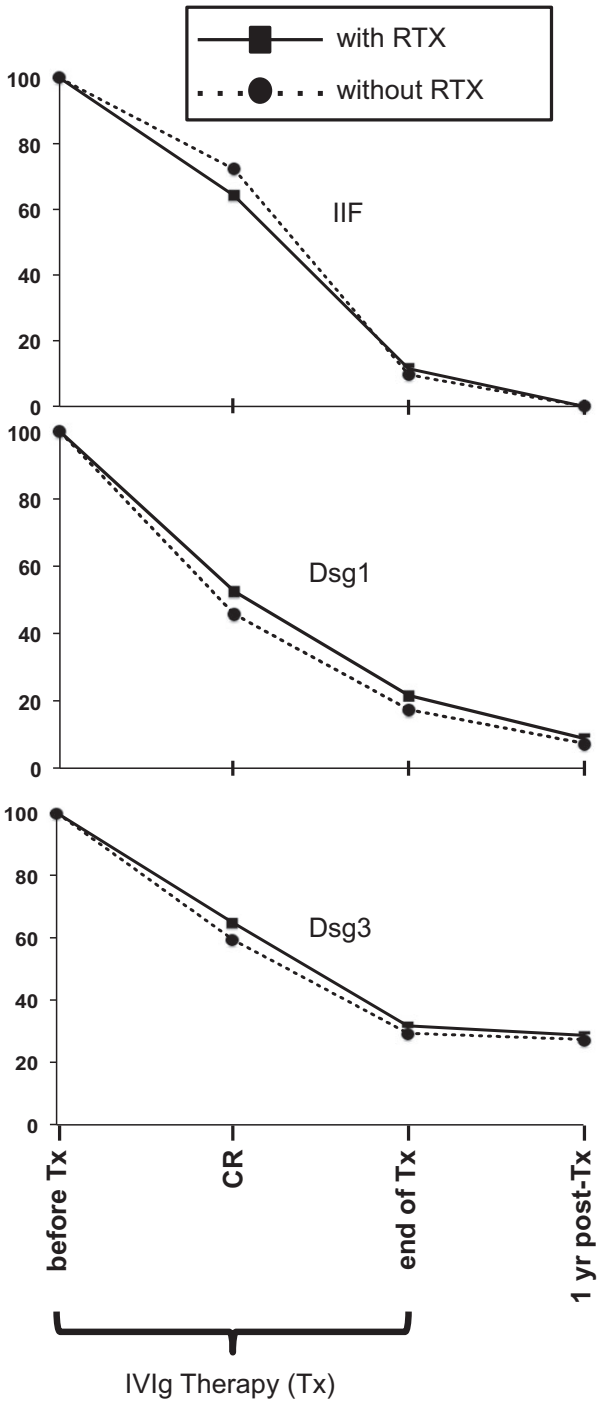

Figure 3 Percentile decrease of the number of pemphigus patients with positive indirect immunofluorescence (IIF) and desmoglein (Dsg) 1 and 3 antibody ELISA results during and after IVIg therapy with or without RTX. Based on the cut-off values of reference laboratory, the positivity of IIF was considered to be at the titer 1/20 and above and that of anti-Dsg 1 and 3 antibodies, 20 units and above. CR = clinical remission, that is, when the PDAAI value became equal to zero.

patient during the entire treatment duration should have received a total of approximately 7 g of prednisone, including approximately 4 g of prednisone administered during TCR. Such dose is consistent with that reported in the literature for prednisone monotherapy during TCR.[28]

A relatively small relapse rate of 12% eliminated the need for additional courses of systemic corticosteroids, allowing to avoid most typical adverse effects of long-term systemic corticosteroid therapy. This was achieved by designing the regimen that optimizes treatment efficacy due to synergy of the drugs included in the treatment protocol. Indeed, each drug had been used in the past for treatment of pemphigus patients but not in such unique combination. Although the absence of control group of patients was a limitation, this was not a clinical trial requiring statistical analysis to prove drug efficacy. Our results were compared to historical controls, that is, clinical trials or observational studies of case series of ≥10 patients reported in the literature, which is a standard approach for the studies of patients with a rare (orphan) disease such as PV and PF.[29,30]

The IVIg dose was based on the reports about the most effective doses of IVIg and the benefit of combining IVIg with an ICD. A trial of different doses of IVIg ranging from 0.5 to 2 g/kg per cycle and different infusion schedules has determined the optimal dose of 2 g/kg per cycle and frequency of cycles under 4 weeks.[31] This frequency is apparently mandated by the need for maintaining the therapeutic dose of IVIg, which depends on the IVIg half-life of ~3 weeks.[32,33] In turn, an increased efficacy of the higher IVIg dose can be explained based on the fundamental principles of IVIg action (reviewed in[7]). The rationale for concurrent administration of IVIg and an ICD was based on observations that, on one hand, depletion of pathogenic antibodies brings about a reciprocal increase in their serum levels, potentially leading to a flare of the disease, and, on the other hand, agents that suppress antibody synthesis can prevent such rebound and thus improve the effectiveness of IVIg therapy of pemphigus.[8,34] The negative feedback mechanism limits the effectiveness of any antibody-eliminating therapy and helps explain sudden disease exacerbation within 2 weeks of IVIg therapy without ICD.[35] This same physiological mechanism, however, provides an opportunity to selectively suppress production of pathogenic antibodies, because only antibodies that have been depleted are resynthesized. The plasma cell producing depleted antibodies thus become a selective target for cytotoxic agents, because the ICDs such as azathioprine and mycophenolate mofetil were shown to be highly efficient against activated, compared to quiescent, lymphocytes.[36,37] Hence, coadministration of IVIg and an ICD leads to "suicidal" proliferation of pathogenic antibody-producing cells. Indeed, it has been documented that pemphigus patients treated with such combined regimen had a greater decline in pathogenic autoantibodies, faster clinical improvement, and required a smaller cumulative dose of systemic corticosteroids.[8,9]

TDC in our patients was only 7-9 days, which apparently resulted from the additive keratinocyte-protecting actions of prednisone/methylprednisolone, minocycline (doxycycline), niacinamide (nicotinamide), and IVIg. The well-known rapid therapeutic effect of high doses of systemic corticosteroids[38–40] is mediated, in part, by their direct anti-acantholytic activity demonstrated *in vitro* using the active glucocorticosteroids hydrocortisone (i.e, synthetic cortisol) and methylprednisolone.[41–43] The inert prodrug prednisone has a lower risk for gastric damage compared to the active drug methylprednisolone[44] and, therefore, is the oral corticosteroid of choice for treating pemphigus patients. Prednisone undergoes its hepatic first pass activation by the enzyme 11β-hydroxysteroid dehydrogenase type 1.[45] Since all patients who were switched to methylprednisolone due to unresponsiveness to high doses of prednisone showed adequate treatment response, one may speculate that nonresponders to prednisone had a lower activity of this enzyme.

Minocycline and niacinamide also exhibit direct anti-acantholytic effects owing to their mitochondrion-protecting properties.[46] The combination of niacinamide and minocycline has been empirically shown to be effective steroid-sparing agents in pemphigus (reviewed in[47]). In addition to their well-known mechanism of pharmacological action, both drugs exhibit the mitochondrion-protecting effects by protecting the mitochondrial oxidative-phosphorylation function (niacinamide) and the outer membrane impermeability (minocycline), both of which are altered by anti-mitochondrial antibodies produced in PV patients.[48]

The average TCR in our patients, 1.7 months, was comparable to that in other pemphigus patients treated by IVIg with or without RTX, which ranges from 1.7 to 2.1 months.[49,50] In marked contrast, TCR in patients treated with RTX without IVIg and patients treated without either IVIg or RTX is much longer 2.3–5.8 months[51,52] (reviewed in[53]) and 4.3–5.9 months,[28,54] respectively. This fact vividly demonstrated the benefit of including IVIg in the treatment protocol. Although we used the internationally agreed upon definition of TCR,[11] there may be some discordance with results reported by other groups.

In an *in vitro* model of therapeutic action of IVIg in pemphigus, normal human IgG has protected cultured keratinocytes from the PV IgG-induced cell death via apoptosis and oncosis, both of which can cause acantholysis.[55] Furthermore, IVIg can facilitate selective elimination of pathogenic antibodies from patients' blood.[56,57]

The most important outcome of the use of our multidrug treatment protocol was a relatively small relapse rate of 12%, which is much lower than that reported for pemphigus patients treated without IVIg (see Table 1 for details). In one of the previous studies, no patient was observed to have had a relapse of pemphigus from IVIg therapy.[31] For comparison, while 90–95% of patients treated with RTX experienced clinical remission, 16–80% had a relapse or required additional RTX (reviewed in[6,28,58–62]). In the most recent studies, relapses occurred in eight out of nine and three out of five patients treated with RTX.[63,64] Duration to complete remission off RTX reported in 22 publications ranged from 2 to 59 months (reviewed in[65]). Notably, 57 (46.3%) of our patients have been followed up for 5 and more years after initiation of treatment. The maximum duration of relapse-free follow-up in our patients is currently 8.1 years (97 months), whereas that reported in the literature is approximately 10 years.[49]

The concurrent administration of IVIg and RTX eliminates the major concern of using RTX in the treatment of pemphigus, such as the high incidence of systemic infections that can lead to fatal septicemia.[66,67] As documented in this study and reported in literature,[49,50] combination of RTX with IVIg brings RTX-related mortality down to zero.

A minor discrepancy between IIF and Dsg 1/3 ELISA results (Fig. 3) was not surprising. Herein, it should be clarified that in addition to anti-Dsg 1 and 3 antibodies, the pool of anti-keratinocyte antibodies produced in pemphigus patients includes a number of non-Dsg autoantibody species, which provides the basis for the multipathogenic explanation of pemphigus pathophysiology.[68] Both anti-Dsg and non-Dsg antibodies are pathogenic in a sense that they are elements of the multifactorial mechanism of keratinocyte damage in pemphigus (apoptolysis[69]). As summarized in a recent viewpoint paper,[68] if non-Dsg antibodies alone were responsible for some cases of PV, one would expect to see a certain number of cases of acute PV with anti-keratinocyte antibodies detectable by direct immunofluorescence and/or IIF but negative by Dsg 1/3 ELISA. This was indeed the case in a number of studies. Different authors reported from 5 up to 33% of PV patients lacking both anti-Dsg 1 and anti-Dsg 3 antibodies by ELISA (reviewed in[68]). Therefore, our results are in keeping with literature reports and altogether suggest that testing the autoantibody response to patient's treatment by IIF is superior to that by ELISA. On the other hand, despite the fact that anti-Dsg antibodies are undetectable in approximately 10% of acute pemphigus patients (reviewed in[68]), the anti-Dsg 1/3 antibody testing by ELISA is useful for initial diagnosis of acute PV and PF.

Conclusions

The retrospective analysis of treatment outcomes in 123 pemphigus patients who received the multidrug treatment regimen combining IVIg with an ICD and mitochondrion-protecting drugs demonstrated that all realistic goals of modern approaches to pemphigus therapy have been met in approximately 88% of patients. The achieved therapeutic goals are the following:

1 Abrupt cessation of development of new and rapid healing of existing lesions;
2 Prompt disappearance of the functional impairment associated with the disease;
3 Complete clinical remission;
4 Prevention of flares; and
5 Lack of serious side effects.

The most important outcome is improvement of the quality of life of the patients. As such, this regimen appears to be superior to the treatment modalities that do not include IVIg. A reasonable expectation that current complete remission in 100% of patients will extend indefinitely makes patients less apprehensive about possible flares and the need for additional therapy. Indeed, the possibility that our patients develop a relapse in the future cannot be completely ruled out. However, since in the vast majority of patients pemphigus usually flares during the first 2 years,[70] the fact that by now 83 and 57 our patients have been in drug-free complete clinical remission for >2 and >5 years, respectively, gives a strong hope that they have been cured. A combination of IVIg and RTX may reverse autoimmunity.[78] The efficacy of the multidrug IVIg protocol does not depend on serological and clinical differences of individual patients, because a combination of IVIg with an ICD eliminates all kinds of pathogenic autoantibodies equally efficiently, and the mitochondrion-protecting drugs uniformly prevent keratinocyte apoptolysis triggered through various autoantibody-activated signaling pathways. Future head-to-head prospective studies should ultimately clarify efficacies of different therapies in defined groups of patients.

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References

1 Ahmed AR, Moy R. Death in pemphigus. *J Am Acad Dermatol* 1982; **7**: 221–228.
2 Rosenberg FR, Sanders S, Nelson CT. Pemphigus: a 20-year review of 107 patients treated with corticosteroids. *Arch Dermatol* 1976; **112**: 962–970.
3 Daoud YJ, Amin KG. Comparison of cost of immune globulin intravenous therapy to conventional immunosuppressive therapy in treating patients with autoimmune mucocutaneous blistering diseases. *Int Immunopharmacol* 2006; **6**: 600–606.
4 Hsu DY, Brieva J, Sinha AA, *et al.* Comorbidities and inpatient mortality for pemphigus in the U.S.A. *Br J Dermatol* 2016; **174**: 1290–1298.
5 Martin LK, Werth VP, Villaneuva EV, *et al.* A systematic review of randomized controlled trials for pemphigus vulgaris and pemphigus foliaceus. *J Am Acad Dermatol* 2011; **64**: 903–908.
6 Colliou N, Picard D, Caillot F, *et al.* Long-term remissions of severe pemphigus after rituximab therapy are associated with prolonged failure of desmoglein B cell response. *Sci Transl Med* 2013; **5**: 175ra30.
7 Amber KT, Shiu J, Ferris K, *et al.* Chapter 39: Role of intravenous immunoglobulin in dermatologic disorders. In: Yamauchi P, ed. *Biologic and Systemic Agents in Dermatology*. Basel: Springer International Publishing AG, 2018: 401–423.
8 Lolis M, Toosi S, Czernik A, *et al.* Effect of intravenous immunoglobulin with or without cytotoxic drugs on pemphigus intercellular antibodies. *J Am Acad Dermatol* 2011; **64**: 484–489.
9 Czernik A, Toosi S, Bystryn JC, *et al.* Intravenous immunoglobulin in the treatment of autoimmune bullous dermatoses: An update. *Autoimmunity* 2012; **45**: 111–118.
10 Rosenbach M, Murrell DF, Bystryn JC, *et al.* Reliability and convergent validity of two outcome instruments for pemphigus. *J Invest Dermatol* 2009; **129**: 2404–2410.
11 Murrell DF, Dick S, Ahmed AR, *et al.* Consensus statement on definitions of disease, end points, and therapeutic response for pemphigus. *J Am Acad Dermatol* 2008; **58**: 1043–1046.
12 Grando SA, Grando AA, Glukhenky BT, *et al.* History and clinical significance of mechanical symptoms in blistering dermatoses: a reappraisal. *J Am Acad Dermatol* 2003; **48**: 86–92.
13 Lever WF *Pemphigus and Pemphigoid*. Springfield: Charles C. Thomas, 1965. p. 1–266.
14 Lever WF, Schaumburg-Lever G. Treatment of pemphigus vulgaris. Results obtained in 84 patients between 1961 and 1982. *Arch Dermatol* 1984; **120**: 44–47.
15 Czernik A, Bystryn JC. Improvement of intravenous immunoglobulin therapy for bullous pemphigoid by adding immunosuppressive agents: marked improvement in depletion of circulating autoantibodies. *Arch Dermatol* 2008; **144**: 658–661.
16 Ahmed AR, Spigelman Z, Cavacini LA, *et al.* Treatment of pemphigus vulgaris with rituximab and intravenous immune globulin. *N Engl J Med* 2006; **355**: 1772–1779.
17 Cremniter D, Baudin M, Roujeau JC, *et al.* Stressful life events as potential triggers of pemphigus [letter]. *Arch Dermatol* 1998; **134**: 1486–1487.
18 Tamir A, Ophir J, Brenner S. Pemphigus vulgaris triggered by emotional stress [letter]. *Dermatology* 1994; **189**: 210.
19 Morell-Dubois S, Carpentier O, Cottencin O, *et al.* Stressful life events and pemphigus. *Dermatology* 2008; **216**: 104–108.
20 Rodan KP, Fleischmann H, Nickoloff BJ, *et al.* Generalized blistering eruption aggravated by heat. Pemphigus foliaceus. *Arch Dermatol* 1987; **123**: 397–398.
21 Ruocco E, Aurilia A, Ruocco V. Precautions and suggestions for pemphigus patients. *Dermatology* 2001; **203**: 201–207.
22 Igawa K, Matsunaga T, Nishioka K. Involvement of UV-irradiation in pemphigus foliaceus. *J Eur Acad Dermatol Venereol* 2004; **18**: 216–217.
23 Wohl Y, Brenner S. Pemphigus in Israel–an epidemiologic analysis of cases in search of risk factors. *Isr Med Assoc J* 2003; **5**: 410–412.
24 Kaplan RP, Potter TS, Fox JN. Drug-induced pemphigus related to angiotensin-converting enzyme inhibitors. *J Am Acad Dermatol* 1992; **26**: 364–366.
25 Kuechle MK, Hutton KP, Muller SA. Angiotensin-converting enzyme inhibitor-induced pemphigus: three case reports and literature review. *Mayo Clin Proc* 1994; **69**: 1166–1171.
26 Fellner MJ, Mark AS. Penicillin- and ampicillin-induced pemphigus vulgaris. *Int J Dermatol* 1980; **19**: 392–393.
27 Duhra P, Foulds IS. Penicillin-induced pemphigus vulgaris [letter]. *Br J Dermatol* 1988; **118**: 307.
28 Chaidemenos G, Apalla Z, Koussidou T, *et al.* High dose oral prednisone vs. prednisone plus azathioprine for the treatment of oral pemphigus: a retrospective, bi-centre, comparative study. *J Eur Acad Dermatol Venereol* 2011; **25**: 206–210.
29 Griggs RC, Batshaw M, Dunkle M, *et al.* Clinical research for rare disease: opportunities, challenges, and solutions. *Mol Genet Metab* 2009; **96**: 20–26.

30 Behera M, Kumar A, Soares HP, *et al.* Evidence-based medicine for rare diseases: implications for data interpretation and clinical trial design. *Cancer Control* 2007; **14**: 160–166.
31 Ahmed AR. Intravenous immunoglobulin therapy in the treatment of patients with pemphigus vulgaris unresponsive to conventional immunosuppressive treatment. *J Am Acad Dermatol* 2001; **45**: 679–690.
32 Saxon A, Stevens RH, Ramer SJ, *et al.* Glucocorticoids administered *in vivo* inhibit human suppressor T lymphocyte function and diminish B lymphocyte responsiveness in *in vitro* immunoglobulin synthesis. *J Clin Invest* 1978; **61**: 922–930.
33 Souders CA, Nelson SC, Wang Y, *et al.* A novel *in vitro* assay to predict neonatal Fc receptor-mediated human IgG half-life. *MAbs* 2015; **7**: 912–921.
34 Bystryn J-C, Jiao D. IVIg selectively and rapidly decreases circulating pathogenic autoantibodies in pemphigus vulgaris. *Autoimmunity* 2006; **39**: 601–607.
35 Messer G, Sizmann N, Feucht H, *et al.* High-dose intravenous immunoglobulins for immediate control of severe pemphigus vulgaris. *Br J Dermatol* 1995; **133**: 1014–1016.
36 Thomas CW, Myhre GM, Tschumper R, *et al.* Selective inhibition of inflammatory gene expression in activated T lymphocytes: a mechanism of immune suppression by thiopurines. *J Pharmacol Exp Ther* 2005; **312**: 537–545.
37 Chaigne-Delalande B, Guidicelli G, Couzi L, *et al.* The immunosuppressor mycophenolic acid kills activated lymphocytes by inducing a nonclassical actin-dependent necrotic signal. *J Immunol* 2008; **181**: 7630–7638.
38 Werth VP. Treatment of pemphigus vulgaris with brief, high-dose intravenous glucocorticoids. *Arch Dermatol* 1996; **132**: 1435–1439.
39 Roujeau JC. Pulse glucocorticoid therapy. The 'big shot' revisited. *Arch Dermatol* 1996; **132**: 1499–1502.
40 Chryssomallis F, Dimitriades A, Chaidemenos GC, *et al.* Steroid-pulse therapy in pemphigus vulgaris long term follow-up. *Int J Dermatol* 1995; **34**: 438–442.
41 Swanson DL, Dahl MV. Methylprednisolone inhibits pemphigus acantholysis in skin cultures. *Journal of Investigative Dermatology* 1983; **81**: 258–260.
42 Jeffes EW, Kaplan RP, Ahmed AR. Acantholysis produced *in vitro* with pemphigus serum: hydrocortisone inhibits acantholysis, while dapsone and 6-mercaptopurine do not inhibit acantholysis. *J Clin Lab Immunol* 1984; **4**: 359–363.
43 Nguyen VT, Arredondo J, Chernyavsky AI, *et al.* Pemphigus vulgaris IgG and methylprednisolone exhibit reciprocal effects on keratinocytes. *J Biol Chem* 2004; **279**: 2135–2146.
44 Metz LM, Sabuda D, Hilsden RJ, *et al.* Gastric tolerance of high-dose pulse oral prednisone in multiple sclerosis. *Neurology* 1999; **53**: 2093–2096.
45 Jenkins JS, Sampson PA. Conversion of cortisone to cortisol and prednisone to prednisolone. *Br Med J* 1967; **2**: 205–207.
46 Chen Y, Chernyavsky A, Webber RJ, *et al.* Critical Role of the Neonatal Fc Receptor (FcRn) in the Pathogenic Action of Antimitochondrial Autoantibodies Synergizing with Anti-desmoglein Autoantibodies in Pemphigus Vulgaris. *J Biol Chem* 2015; **290**: 23826–23837.
47 McCarty M, Fivenson D. Two decades of using the combination of tetracycline derivatives and niacinamide as steroid-sparing agents in the management of pemphigus: defining a niche for these low toxicity agents. *J Am Acad Dermatol* 2014; **71**: 475–479.
48 Kalantari-Dehaghi M, Chen Y, Deng W, *et al.* Mechanisms of mitochondrial damage in keratinocytes by pemphigus vulgaris antibodies. *J Biol Chem* 2013; **288**: 16916–16925.
49 Ahmed AR, Kaveri S, Spigelman Z. Long-term remissions in recalcitrant pemphigus vulgaris. *N Engl J Med* 2015; **373**: 2693–2694.
50 Ahmed AR, Nguyen T, Kaveri S, *et al.* First line treatment of pemphigus vulgaris with a novel protocol in patients with contraindications to systemic corticosteroids and immunosuppressive agents: Preliminary retrospective study with a seven year follow-up. *Int Immunopharmacol* 2016; **34**: 25–31.
51 Kim TH, Choi Y, Lee SE, *et al.* Adjuvant rituximab treatment for pemphigus: a retrospective study of 45 patients at a single center with long-term follow up. *J Dermatol* 2017; **44**: 615–620.
52 Uzun S, Bilgic Temel A, Akman Karakas A, *et al.* Efficacy and safety of rituximab therapy in patients with pemphigus vulgaris: first report from Turkey. *Int J Dermatol* 2016; **55**: 1362–1368.
53 Wang HH, Liu CW, Li YC, *et al.* Efficacy of rituximab for pemphigus: a systematic review and meta-analysis of different regimens. *Acta Derm Venereol* 2015; **95**: 928–932.
54 Benoit Corven C, Carvalho P, Prost C., *et al.* [Treatment of pemphigus vulgaris by azathioprine and low doses of prednisone (Lever scheme)]. *Ann Dermatol Venereol* 2003; **130**: 13–15.
55 Arredondo J, Chernyavsky AI, Karaouni A, *et al.* Novel mechanisms of target cell death and survival and of therapeutic action of IVIg in pemphigus. *Am J Pathol* 2005; **167**: 1531–1544.
56 Czernik A, Beutner EH, Bystryn JC. Intravenous immunoglobulin selectively decreases circulating autoantibodies in pemphigus. *J Am Acad Dermatol* 2008; **58**: 796–801.
57 Bystryn JC, Jiao D, Natow S. Treatment of pemphigus with intravenous immunoglobulin. *J Am Acad Dermatol* 2002; **47**: 358–363.
58 Almugairen N, Hospital V, Bedane C, *et al.* Assessment of the rate of long-term complete remission off therapy in patients with pemphigus treated with different regimens including medium- and high-dose corticosteroids. *J Am Acad Dermatol* 2013; **69**: 583–588.
59 Ahmed AR, Shetty S. A comprehensive analysis of treatment outcomes in patients with pemphigus vulgaris treated with rituximab. *Autoimmun Rev* 2015; **14**: 323–331.
60 Sharma VK, Bhari N, Gupta S, *et al.* Clinical efficacy of rituximab in the treatment of pemphigus: a retrospective study. *Indian J Dermatol Venereol Leprol* 2016; **82**: 389–394.
61 Atzmony L, Hodak E, Leshem YA, *et al.* The role of adjuvant therapy in pemphigus: a systematic review and meta-analysis. *J Am Acad Dermatol* 2015; **73**: 264–271.
62 Ellebrecht CT, Choi EJ, Allman DM, *et al.* Subcutaneous Veltuzumab, a Humanized Anti-CD20 Antibody, in the Treatment of Refractory Pemphigus Vulgaris. *JAMA Dermatol* 2014; **150**: 1331–1335.
63 Cho YT, Lee FY, Chu CY, *et al.* First-line combination therapy with rituximab and corticosteroids is effective and safe for pemphigus. *Acta Derm Venereol* 2014; **94**: 472–473.
64 Ingen-Housz-Oro S, Valeyrie-Allanore L, Cosnes A, *et al.* First-line treatment of pemphigus vulgaris with a combination of rituximab and high-potency topical corticosteroids. *JAMA Dermatol* 2015; **151**: 200–203.
65 Cholera M, Chainani-Wu N. Management of Pemphigus Vulgaris. *Adv Ther* 2016; **33**: 910–958.

66 Gurcan HM, Keskin DB, Stern JN, *et al.* A review of the current use of rituximab in autoimmune diseases. *Int Immunopharmacol* 2009; **9**: 10–25.
67 El Tal AK, Posner MR, Spigelman Z, *et al.* Rituximab: a monoclonal antibody to CD20 used in the treatment of pemphigus vulgaris. *J Am Acad Dermatol* 2006; **55**: 449–459.
68 Ahmed AR, Carrozzo M, Caux F, *et al.* Monopathogenic vs. multipathogenic explanations of pemphigus pathophysiology. *Exp Dermatol* 2016; **25**: 839–846.
69 Grando SA, Bystryn JC, Chernyavsky AI, *et al.* Apoptolysis: a novel mechanism of skin blistering in pemphigus vulgaris linking the apoptotic pathways to basal cell shrinkage and suprabasal acantholysis. *Exp Dermatol* 2009; **18**: 764–770.
70 Pasricha JS, Khaitan BK. Curative treatment for pemphigus. *Arch Dermatol* 1996; **132**: 1518–1519.
71 Daniel BS, Hertl M, Werth VP, *et al.* Severity score indexes for blistering diseases. *Clin Dermatol* 2012; **30**: 108–113.
72 Herbst A, Bystryn JC. Patterns of remission in pemphigus vulgaris. *J Am Acad Dermatol* 2000; **42**: 422–427.
73 Kavusi S, Daneshpazhooh M, Farahani F, *et al.* Outcome of pemphigus vulgaris. *J Eur Acad Dermatol Venereol* 2008; **22**: 580–584.
74 Uzun S, Durdu M, Akman A, *et al.* Pemphigus in the Mediterranean region of Turkey: a study of 148 cases. *Int J Dermatol* 2006; **45**: 523–528.
75 Harman KE, Albert S, Black MM, *et al.* Association of. Guidelines for the management of pemphigus vulgaris. *Br J Dermatol* 2003; **149**: 926–937.
76 Alexandroff AB, Harman KE. Blistering skin disorders: an evidence-based update. Conference report. *Br J Dermatol* 2009; **160**: 502–504.
77 Amber KT, Hertl M. An assessment of treatment history and its association with clinical outcomes and relapse in 155 pemphigus patients with response to a single cycle of rituximab. *J Eur Acad Dermatol Venereol* 2015; **29**: 777–782.
78 Ahmed AR, Kaveri S. Reversing autoimmunity combination of rituximab and intravenous immunoglobulin. *Front Immunol* 2018; **9**: 1189.

