11

CHAPTER 11
Discussion and future perspectives

Aniek Lamberts

Center for Blistering Diseases, Department of Dermatology,
University Medical Center Groningen, University of Groningen,
Groningen, the Netherlands

PART 1 : Nonbullous pemphigoid: disease characteristics and immunological aspects

Pemphigoid-specific autoantibodies in healthy individuals

Several studies have detected pemphigoid-specific IgG autoantibodies in the serum of nondiseased people.[1–3] In chapter 2 we assessed the presence of circulating pemphigoid-specific autoantibodies in dermatology patients with a nonbullous skin disorder. A diagnosis of pemphigoid was excluded based on skin biopsy with negative direct immunofluorescence microscopy. Single serological test positivity was found in 14% of the patients, whom had a higher median age compared to patients with negative results. In ten patients (4%), multiple serologic tests were positive. Interestingly, in chapter 6 we also observed circulating IgE autoantibodies to NC16A (8%) and BP230 (20%) in elderly controls with pruritus by enzyme-linked immunosorbent assay (ELISA).

The detection of pemphigoid-specific autoantibodies by ELISA might partly be a result of nonspecific binding, however, it is also hypothesized that such autoantibodies can develop due to a combination of epitope spreading and aging of the immune system. Epitope spreading is previously defined as a specific autoreactive immune response to endogenous epitopes on proteins, secondary to the release of such self-protein during a chronic autoimmune, or inflammatory response.[3] Epitope spreading occurs in several autoimmune diseases, including early in BP.[3–5] Possibly, immunologically “hidden” epitopes are “revealed” due to cell injury in patients with chronic pruritus by scratching. A second potentially contributing factor is aging of the immune system, which is associated with elevated levels of pro-inflammatory cytokines, and a decline of naive regulatory T cells (Tregs).[6,7] Interestingly, a decline of Tregs in blood and skin of BP patients was reported previously.[8] Moreover, scurfy mice and patients with IPEX (immunodysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, whom both lack functional Tregs, spontaneously produced autoantibodies against several antigens located in the BMZ.[9–11] This suggests that functional Tregs are essential to prevent self-reactivity to BMZ proteins.

Based on chapter 2, an important question arose; “What are the minimal requirements for a diagnosis of bullous and nonbullous forms of pemphigoid?”. In 2019, Meijer *et al*. addressed this clinical dilemma in a diagnostic accuracy study, and provided minimal diagnostic criteria for BP and NBP.[12] In line with previous

findings, IIF SSS showed highest specificity (99.9%), and a positive predictive value of 99.6% for the diagnosis of pemphigoid.[12,13] ELISA had no diagnostic value, but was recommended for monitoring disease activity in confirmed pemphigoid patients. Their findings were translated into a 2-out-of-3 rule for the diagnosis of pemphigoid, meaning patients need to fulfill two of the three following criteria: 1) compatible clinical presentation with pruritus and/or predominant skin blisters, 2) positive linear IgG and/or C3c staining along the BMZ by DIF, 3) positive IgG staining on the epidermal side of SSS by IIF. When applying the 2-out-of-3 rule in retrospect, ten patients in chapter 2 fulfilled the diagnostic criteria, and thus received a delayed diagnosis of NBP. Unpublished data regarding these ten patients revealed pruritus in all, and multiple positive serologic tests with predominant BP230 reactivity. It is unknown whether nondiseased patients with pemphigoid-specific autoantibodies detected by ELISA in chapter 2 and 6 are at risk to develop pemphigoid in the future. Long-term follow-up of these patients could answer this question.

Disease characteristics of nonbullous pemphigoid

The prevalence of nonbullous pemphigoid

The NBP phenotype was reported in approximately 20% of the BP cases, but the prevalence of NBP was not studied before.[12,14,15] In chapter 5, we assessed the prevalence of pemphigoid, bullous and nonbullous, in a high-risk population of nursing home residents. We observed an exceptional high pemphigoid prevalence of 6%. In 2014, an estimated BP prevalence of 0.026% was reported in the general population of Germany, and 0.3% in elderly people aged above 85 years.[16] One previous study assessed the incidence of BP in nursing home residents, and observed a crude incidence of 5% per year, by using clinical skin blisters combined with histopathology findings of a subepithelial split, or positive DIF as diagnostic criteria for BP.[17] Our study was the first to assess the full spectrum of pemphigoid in a nursing home population by including NBP. Surprisingly, NBP was more prevalent than BP (4 vs. 3 cases), implying that the nonbullous phenotype of pemphigoid may be more common as previously assumed.[18] Importantly, all four NBP cases were misdiagnosed by the nursing home physician with other pruritic skin diseases. This highlights the need for more awareness for NBP, especially amongst healthcare specialists whom care for elderly populations at risk to develop pemphigoid.

Interestingly, all seven identified pemphigoid cases had neurodegenerative diseases: dementia in five, Parkinson's disease in one, and cognitive decline in one patient. In line, several studies observed that neurodegenerative diseases often precede pemphigoid.[19–21] The expression of BP180 and BP230 in both skin and brain tissue suggests that cross-reactivity could initiate pemphigoid.[21,22] Recently, autoantibodies to BP180 were detected in a large number of patients with Alzheimer's disease and multiple sclerosis (20% and 54%), however, the autoantibodies were directed against nonpathogenic intracellular and mid-extracellular domains of BP180.[23,24] Further studies need to identify factors that trigger intra- and intermolecular epitope spreading, and subsequently initiate pemphigoid disease.

Clinical presentation of nonbullous pemphigoid

In chapter 3 and 4, we explored the disease characteristics of NBP by systematic review, and a retrospective case study. Corresponding findings were disease onset at old age (75 vs. 76 years), and the heterogeneous clinical presentation with a variety of skin lesions. Both chapters reported nonspecific histopathologic findings in NBP, and blister development during follow-up in the minority of patients (10% and 17%). In line, a recent case series reporting on 36 NBP patients observed blister formation in only 23%.[25] In chapter 4, NBP patients with late blister formation had a significant longer follow-up period compared to patients that did not develop blisters. Possibly, blisters might have occurred in more NBP patients if their follow-up period was longer. However, the longer follow-up could also be related to the event of developing blisters, since patients with late blister development likely return for additional treatment advices. Nonetheless, multiple NBP cases remained nonbullous during long follow-up periods, up to 14 years. These findings suggest that NBP should be seen as a phenotypic variant within the pemphigoid spectrum, rather than a prodromal phase of BP.

Several results of chapter 3 and 4 did not correspond. First, published NBP cases most often presented with urticarial papules and plaques (52%), while in our NBP cohort papules and nodules were most common (31%). The high number of published NBP patients with urticarial lesions might be explained by a higher resemblance to the typical clinical picture of BP, triggering clinicians to perform pemphigoid diagnostics more easily. Second, published NBP cases were reasonably equally reactive to BP230 (ELISA 53%; immunoblot 56%), and BP180 (ELISA 58%;

immunoblot 32%), while NBP patients in our cohort were predominantly reactive to BP230 (ELISA 47%; immunoblot 41%), and less common to BP180 (ELISA 31%; immunoblot 13%). Possibly, less BP230 reactive NBP cases are published because BP230 reactivity is associated with DIF negativity, and current consensus based guidelines appoint DIF positivity as golden standard for pemphigoid diagnosis.[12,26,27] Recently, the need for DIF positivity is refuted by Meijer *et al*., as was discussed above.[12] Presumably, the less NBP patients resemble the typical BP phenotype and immune profile, the less likely clinicians think of pemphigoid. This could explain the subtle differences between published NBP cases, and our cohort of patients diagnosed in a center with high awareness for NBP, and routine pemphigoid diagnostics performed in elderly patients with itch.

Interestingly, Ben Mordehai *et al*. reported mucosal involvement in 6 of 36 (16.7%) NBP cases.[25] In contrast, in chapter 3 and 4 we observed mucosal involvement in only one NBP case published by Ameen et al.[28] We question whether patients with bullous or erosive mucosal lesions should receive the diagnosis NBP, as per definition they are not nonbullous. Clinicians must be cautious, and should consider a diagnosis of MMP in patients with nonbullous pruritic skin lesions and mucosal involvement.

Immunological aspects of nonbullous pemphigoid

An important question is: "Why do NBP patients not develop blisters?". Although the data in this thesis does not provide the answer, we highlight and discuss potential clues below.

Predominant BP230 reactivity in nonbullous pemphigoid

In chapter 4, autoantibodies in NBP were predominantly BP230 reactive, opposed to autoantibodies in BP that commonly target BP180, previously described by Meijer *et al*.[12] The pathogenicity of anti-NC16A autoantibodies was proven repeatedly in animal models, and correlated with disease activity in humans.[29–33] The pathogenicity of autoantibodies to intracellular BP230 was initially debated, as autoantibodies to BP230 did not lead to skin blistering in mouse studies,[34,35] and did not correlate with disease activity in BP patients.[30,31,36] Therefore, authors concluded that anti-BP230 autoantibodies must be nonpathogenic.

Nevertheless, other studies did find evidence for a pathogenic role of anti-BP230 autoantibodies. Hall III *et al*. were one of the first, and induced an anti-

BP230 autoantibody response in rabbits, that showed no clinical evidence of skin disease at first.[37] However, epithelial injury by ultraviolet B radiation led to an enhanced immune response with epidermal necrosis and linear IgG and C3 deposits at the BMZ, suggesting the intracellular BP230 antigen must be exposed before disease initiates.[37] A second study by Kiss *et al*. subcutaneously injected anti-BP230 autoantibodies into the dorsal skin of seven mice, which induced macroscopic blistering in only one.[38] However, all seven mice had erythematous skin, clinically resembling NBP, with histological subepithelial blisters, IgG and C3 depositions along the BMZ, and an intradermal inflammatory reaction. More recently, two studies reported spontaneous anti-BP230 autoantibodies in scurfy mice that lack Tregs.[9,10] The mice displayed a blistering pemphigoid phenotype in one study, and eczematous lesions in the other. Haeberle *et al*. proved the pathogenicity of anti-BP230 autoantibodies by transferring them into neonatal mice, where they induced subepithelial blisters.[9] The animal studies discussed above showed evidence that anti-BP230 autoantibodies have pathogenic potential in animals. But what about humans?

In epidermolysis bullosa simplex, homozygous nonsense mutations in the BP230 gene resulted in an absent or truncated BP230 protein, with a clinical phenotype consisting of generalized skin fragility, skin blistering, and in one case prurigo papules.[39–41] Moreover, Tanaka *et al*. studied the relation between clinical data and antigen profile in 100 BP patients, of which 44 patients were reactive to BP230 only.[42] These patients needed lower steroid dosages compared with those reactive to BP180 only, however, no differences in disease severity, lesion extent, and steroid responsiveness were observed. Hayawaka *et al*. performed a similar study, and also found a lower need for systemic corticosteroids in patients with anti-BP230 autoantibodies only (anti-BP230 type BP), but also reported a lower disease severity.[43] Moreover, they described 'unique skin lesions' compatible with eczematous pemphigoid, pemphigoid nodularis, vesicular pemphigoid, dyshidrosiform pemphigoid, and pretibial localized pemphigoid, implying some of the clinical phenotypes were nonbullous. In contrast, in chapter 4 we did not observe mild disease in NBP patients, whom were often BP230 reactive, but found a frequent necessity of systemic therapy, and increased mortality rates.

Hayakawa *et al*. additionally mapped the epitopes of patients with anti-BP230 type BP, and showed specific recognition of the C-terminal.[43] In contrast, all three domains of BP230 were targeted in BP patients recognizing both BP180 and BP230,

suggesting these BP230 autoantibodies might develop secondary to intermolecular epitope spreading.[4,43] The authors proposed that anti-BP230 type BP might be a different disease entity, with possibly a different pathogenesis, resulting in a different clinical phenotype.[43] We suggest that NBP can be such a phenotype.

Complement activation in nonbullous pemphigoid

In BP the activation of complement through classical and alternative pathways presumably contributes in blister formation by attraction and activation of immune cells by anaphylatoxins C3a and C5a.[44–46] Two studies reported significantly less complement C3c deposits along the BMZ in NBP patients (39% and 52%), compared to BP patients (83% and 77%).[12,47] In line, in chapter 4 we observed complement deposits along the BMZ in only 21% of NBP cases, and in chapter 7 a lower expression of genes related to complement activation was found in all NBP biopsies, compared to high expression in 60% of BP biopsies. These findings suggest that complement activation could be the missing link to develop blisters in a subset of NBP patients.

Complement activation through autoantibody binding (classical pathway) can be induced by IgG1 and IgG3 subclasses that have high complement activating abilities, whereas IgG2 has low, and IgG4 has no complement activating abilities.[48] In BP, autoantibodies commonly exist of IgG1 and IgG4 subclasses.[33,49–51] The lower prevalence of complement deposits in NBP skin suggests that IgG2 or IgG4 might be involved. In line, Lamb *et al*. found predominant IgG4 subclass positivity in 30 patients with 'prodromal BP'.[52] Zheng *et al*. assessed IgG subclasses of six patients with anti-BP230 type BP, and found IgG4 positivity in all, while IgG1 and IgG3 were faint or negative.[53] Eleven patients with autoantibodies against both BP180 and BP230 also showed IgG4 subclasses (82%), however, together with IgG1 (91%) and IgG3 (64%). As expected, complement C3 deposits were weaker in anti-BP230 type BP compared to BP180+BP230 positive BP.

Interestingly, Buschman *et al*. linked IgG4 subclass predominance with false-negative DIF results in twelve BP patients.[54] The authors demonstrated that BP patients with an initial negative DIF actually did have IgG4 deposits along the BMZ, and suggested that IgG4 subclasses might be below the detection threshold of commercial IgG conjugates used in conventional DIF microscopy.[54] However, in this study isotonic salt was not used as biopsy medium, which could have significantly improved the visibility of low IgG4 signals by reducing the overall IgG background

staining. The IgG subclasses of autoantibodies in NBP were not studied in this thesis, but will be topic of future research.

T helper 2 responses in nonbullous pemphigoid

T lymphocytes have a role in initiating and regulating immune responses. T cell responses in NBP were not studied so far, while elevated levels of T helper 1, 2 and 17 (Th1, Th2, Th17) cells and their related cytokines were reported in serum and skin of BP patients.[55–58] Moreover, studies on Th2 responses in BP revealed potential pathogenic roles of eosinophils and IgE, which led to targeted therapies that are currently explored.[59–63]

In chapter 3 and 4 eosinophils were found elevated in the peripheral circulation of NBP (87% and 45%) in similar and higher rates as in BP (50%).[64] In line, Ben Mordehai *et al.* reported a significantly higher blood eosinophil count in NBP compared with BP.[25] Eosinophils were detected in histopathologic skin biopsies in 47% of published NBP cases in chapter 3, and in 69% cases of our NBP cohort in chapter 4. Interestingly, eosinophilic spongiosis was less common in NBP (8% and 6%) compared to reports in BP (50%), implying that eosinophils seldom infiltrate the epidermis in NBP.[65]

We further assessed the presence of pemphigoid-specific IgE autoantibodies in serum and skin of NBP and BP patients in chapter 6, and observed no significant differences. In contrast, Ben Mordehai *et al.* found significantly higher total IgE levels in serum of NBP compared to BP, but did not assess pemphigoid-specific antibody titers.[25] Interestingly, in chapter 6 both NBP and BP skin showed IgE autoantibodies attached to the surface of cells in the dermis, likely eosinophils and mast cells, while IgE deposits in a linear pattern along the BMZ were only found in 2 out of 28 skin biopsies (7%; 1 NBP, 1 BP). The high number of studies that do find linear IgE along the BMZ (in up to 44% of BP biopsies) is surprising, and the conflicting findings must be caused by methodological differences.[66–71] Our results suggest that IgE does not directly mediate blister formation by mast cell and eosinophil degranulation in pemphigoid, but could indirectly influence the disease pathogenesis.

Surprisingly, further assessment of the immunological profile of NBP in chapter 7 revealed a lower expression of Th2 related genes compared with BP. Combining our findings, we may conclude that chapter 3, 4 and 6 provide evidence for Th2 responses in both NBP and BP, however, data of chapter 7 implies that Th2

responses are more extensive in BP than in NBP. Future studies need to examine Th2 responses in NBP, and need to validate the genetic data presented in chapter 7.

Several immunological pathways and immune players that were thought to play a role in the pathogenesis of NBP and BP are summarized in figure 1. Moreover, an overview of diagnostic immunological findings is provided in table 1.

Table 1. Diagnostic and immunological findings in bullous and nonbullous pemphigoid

Diagnostic/immunological tests	Bullous pemphigoid*	Nonbullous pemphigoid
Direct immune fluorescence microscopy		
Positive staining of IgG, %	88%	60%
Positive staining of C3c, %	83%	21%
Positive staining of IgE, %	7%	7%
Serological tests		
Positive IgG NC16A ELISA (cut-off index >9), %	70%	31%
Positive IgG BP230 ELISA (cut-off index >9), %	45%	47%
Positive IgE NC16A ELISA** (cut-off OD value 620), %	18%	9%
Positive IgE BP230 ELISA** (cut-off OD value 929), %	34%	22%
Peripheral blood eosinophilia (> 0.40 10^9/L), %	50%	45%
Elevated total IgE (>115kU/L), %	60%	63%
Predominant IgG subclass	IgG1 and IgG4	IgG4

*ELISA, enzyme-linked immunosorbent assay. *Percentages on diagnostic findings concerning BP were based on literature.[12,64] Percentages concerning diagnostic findings in NBP were based on chapter 4 and 6 of this thesis. Subclass predominance in bullous pemphigoid and nonbullous pemphigoid was based on literature.[50-52] ** The methodology of the IgE ELISAs is described in chapter 6 of this thesis.*

Management and prognosis of nonbullous pemphigoid

Little is published about the management and prognosis of NBP. Lamb *et al*. treated 'prodromal BP' patients with high potent topical steroids (disease resolution in 55%), oral corticosteroids (disease resolution in 27%), tetracycline (disease resolution in 0%), a combination of these three therapies (disease resolution in 50%), or oral corticosteroids combined with an immunosuppressive

drug (unknown which; disease resolution in 75%).[72] Limited follow-up data described relapses within 1 year in 10%, and one death. In line with the study of Lamb *et al.,* chapter 4 showed similar response rates for topical and oral steroids in NBP patients. Moreover, doxycycline monotherapy was also ineffective in our NBP cohort. However, differences in outcome measures in the study of Lamb *et al*. and chapter 4 does not allow us to draw hard conclusions when comparing results. The study of Ben Mordehai *et al.* used consensus defined outcome measures[73] similar to chapter 4, however, they only focused on achievement of disease control, and did not report remission rates on/off therapy.[25] They found sufficient disease control by topical steroids in only 8% and 9% of NBP and BP patients, while all other patients were in need of systemic therapy. Overall, 88% of NBP and 86% of BP patients achieved disease control, but by which treatment was not specified.

Interestingly, our data revealed that NBP responded well to methotrexate (MTX) in a low dose, with long term remission in 43% of the treated patients. Recently, Delaumenie *et al*. analyzed data of BP patients treated with MTX, and observed a higher response in patients with anti-BP230 autoantibodies compared to patients with anti-BP180 autoantibodies (1 year response rate of 75% vs. 35%).[74] Possibly, a difference in disease mechanism in anti-BP230 autoantibody mediated pemphigoid could explain these findings, as was also discussed above.

No previous studies assessed the mortality risk in NBP. In chapter 4, over one-third of the patients in our cohort died within a mean time of 2 years after receiving the diagnosis NBP. The all-cause standardized mortality ratio in the NBP population was 8.6, which is surprisingly higher than reports on BP (3.4 to 6.6).[75–77] However, no hard conclusions could be drawn, since our data was limited by a low sample size. It can be hypothesized that the academic setting, in which it is more likely to have severe symptoms, and the long diagnostic delay without adequate therapy influenced the mortality rate. Nonetheless, our findings imply that NBP could not be set aside as a milder disease variant of BP.

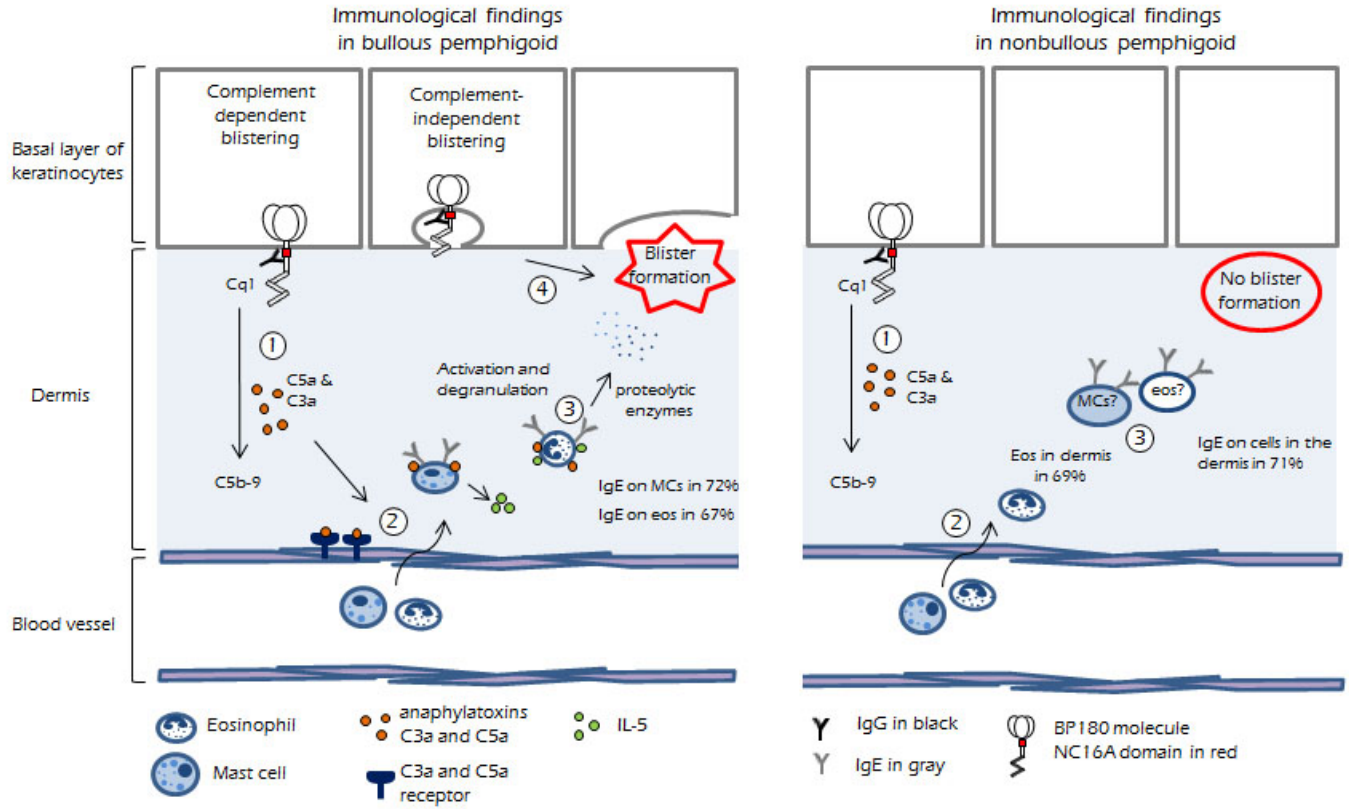

Figure 1. Schematic overview of immunological findings in bullous and nonbullous pemphigoid. *Bullous pemphigoid:* 1. Autoantibody binding leads to complement activation by the classical pathway. 2. Anaphylatoxins C3a and C5a bind receptors at the endothelium, allowing immune cells to migrate into the dermis. 3. Mast cells secrete IL-5, a potent activator of eosinophils. C3a, C5a and IgE bind to mast cells and eosinophils could lead to activation. Activation leads to degranulation of proteolytic enzymes that degrade proteins of the hemidesmosome, causing blister formation. 4. Complement independent blister formation may take place by autoantibody dependent pinocytosis, depleting BP180 from the hemidesmosome, subsequently causing blister formation. *Nonbullous pemphigoid:* 1. In a subset of patients complement activation might occur through the classical pathway, as in 21% C3c can be detected linear along the basement membrane zone by direct immunofluorescence microscopy. 2. In chapter 4, eosinophils are detected in histopathology biopsies in 69%. 3. In chapter 6, IgE was detected on cells in the dermis in 71%, likely eosinophils or mast cells. MCs, mast cells; eos, eosinophils; IL-5, interleukin 5.

Future perspectives

Part 1 of this thesis bundles the first studies that focus on characterizing NBP. In general, NBP is understudied, and many gaps in knowledge exist. The long diagnostic delays, observed in chapter 3 and 4, and frequent misdiagnosis shown in chapter 5, illustrate the high need for more awareness for NBP amongst clinicians. Interestingly, this need was also expressed by others. In chapter 4A, a letter to the editor remarked that NBP was unmentioned in clinical guidelines for chronic pruritus.[78,79] In chapter 8, more awareness for NBP was prioritized as third most important unmet need by 30 of 35 clinicians participating in our survey study. This need was also spontaneously mentioned by 2 of 71 participating patients. How to achieve better disease recognition of NBP by clinicians should be the focus and topic of future discussions. Important steps include more research on the prevalence of NBP, and its disease pathogenesis, which may contribute to more acknowledgement of this disease entity by the medical community, and better therapies.

PART 2: Management of pemphigoid diseases

Unmet needs in pemphigoid diseases

Over the last decades, the role of patients to set the research agenda became more important.[80,81] The James Lind Alliance (JLA) is an organization that supports Research Priority Setting Partnerships, to prevent a mismatch in studies desired by patients and clinicians, and studies actually performed by researchers.[82] In chapter 8, we explored and prioritized unmet needs in pemphigoid diseases by online survey distributed among patients, clinicians and researchers, using a JLA-like methodology. We found that the need for better therapeutic options was ranked most urgent by all interest groups. Patients expressed the need for '*better treatment options*', clinicians for '*labeling of new drugs for the indication of pemphigoid*', and researchers for '*more head-to-head randomized controlled trials comparing effectiveness and safety of current treatments*'. Conventional systemic therapies for pemphigoid diseases include oral corticosteroids, methotrexate, dapsone, mycophenolate mofetil, cyclosporine, cyclophosphamide, azathioprine, and all may give serious short- and long-term side effects. Novel treatments for pemphigoid are currently being evaluated, and we will discuss several below.

B cell targeting therapies

Rituximab

Limited data is available on RTX in pemphigoid diseases, therefore, we analyzed our daily practice data of RTX therapy in 28 patients with recalcitrant pemphigoid diseases in chapter 9. We found an overall remission rate of 57%, with best effects in MMP and BP (remission in 64% and 63%). A recent case series of 20 BP patients treated with RTX (19 with 1000mg on day 1 and 15; 1 with 375mg/m^2 weekly for four weeks) even reported a higher remission rate (75%).[83] Prospective clinical trials need to determine the effectiveness of RTX and its biosimilars in pemphigoid diseases with more certainty. Moreover, further studies are required to evaluate the best dosing schedule.

Only little is known about the changes that B cells undergo during RTX therapy. A recent study analyzed the immunological phenotype of the B cell population in 17 BP patients prior to RTX, and after B cell repopulation.[84] BP180-IgG-positive and BP180-IgM-positive B cells were decreased fivefold by RTX, and only BP180-IgM-positive B cells reappeared in a low frequency at month 24. Interestingly, in these B cells a shift in cytokine pattern was noticed with decreased expression of inflammatory cytokines IL-15 and IL-6. Moreover, two patients with long term complete remission off therapy showed elevated expression of anti-inflammatory cytokines IL-10 and IL-1RA on their B cells, suggesting the switch in cytokine pattern reflects on whether or not patients responded well to RTX. Future studies with similar methodology performed in a larger sample size should aim to identify factors that predict treatment success prior to RTX therapy, so that candidates for this treatment can be preselected.

The safety profile of RTX appeared similar, or more favorable than of conventional therapies.[83,85,86] In chapter 9, one patient developed a pneumocystis pneumonia (PCP), which is a potentially life threatening opportunistic fungal infection caused by *Pneumocystis jiroveci*. In chapter 10 we assessed the incidence of PCP infections in patients with autoimmune bullous diseases without PCP prophylaxis, to determine whether or not standard prophylaxis is warranted. Our observed PCP incidence rate was 0.1%, which was below the threshold of 3.5%, calculated by Green *et al.*, by which the number needed to treat significantly outweighs the number needed to harm.[87] In 2019, Rekhtman *et al*. assessed the PCP incidence in 27 health care organizations, and observed the highest PCP

incidence (0.2%) in patients using an immunosuppressant combined with systemic corticosteroids.[88] By concluding that PCP prophylaxis is warranted in these patients, despite not reaching the 3.5% threshold, the authors launched a discussion wherein it was argued that the calculated threshold of 3.5% did not take severity of the harm into account.[87,89,90] Authors debated that the harm of a PCP infection (mortality rates between 30-60%) is higher compared with trimethoprim-sulfamethoxazole use (mostly laboratory abnormalities resolving with discontinuation, and very low percentage of Stevens-Johnson syndrome). Siscos *et al.* discussed the use of dapsone instead of trimethoprim-sulfamethoxazole for PCP prophylaxis, which provides an additional therapeutic benefit at a low cost.[91] In a response letter, it was commented that trimethoprim-sulfamethoxazole is significantly more effective than dapsone in preventing PCP, limiting its use for the proposed dual purpose.[92] While our data in chapter 10 made it clear that standard prophylaxis is not necessary in patients with autoimmune bullous diseases, the data of Rekhtman *et al.* shows that the level of immunosuppression strongly influences the PCP risk. Larger cohort studies on autoimmune bullous patients that take the amount of immunosuppression into account, may reveal which specific drug combinations are an indicator for PCP prophylaxis.

Other B cells targeting therapies

In chapter 9, RTX appears to be a relatively effective and safe treatment option for pemphigoid diseases, but still, the response rates were lower as in pemphigus diseases.[93] Other drugs targeting B cells are currently tested, including ofatumumab, which also targets CD20, belimumab targeting BAFF (B cell activating factor), and ibrutinib, which is a BTK (Bruton's tyrosine kinase) inhibitor.[94] Another interesting attempt to more specifically deplete autoreactive B cells was proposed, using chimeric antigen receptor therapy to target desmoglein-3 specific B cells in pemphigus vulgaris, leaving the nonautoreactive B cell populations intact.[95] If this strategy is successful, it might also be possible to engineer this drug into a therapy for pemphigoid diseases.

Future perspectives

In the upcoming years, there lies an important task for clinicians and researchers to address the need for better therapeutic options for pemphigoid diseases. This need can be addressed by different approaches. Drugs that have shown beneficial

effects in other autoimmune diseases with common immunological pathophysiology can be tested. Moreover, a better understanding of the pathophysiology of pemphigoid diseases may identify potential targets to develop novel therapies. A number of promising drugs are currently under investigation.

Complement activation has been designated as an important event in the disease mechanism of pemphigoid diseases. Currently, several clinical trials on anti-complement drugs in pemphigoid diseases are ongoing, and their results are expected soon. Beside complement, IgE and eosinophils were also suggested to play an important role in the pathophysiology. The anti-IgE drug omalizumab, registered for chronic urticaria, has been successful in several preselected BP patients, however a suboptimal long term effectiveness was observed.[63] Recent studies demonstrated IgE autoantibodies in serum and skin of EBA and MMP patients, suggesting that omalizumab could be effective in other pemphigoid diseases as well.[96–99] Other drugs of interest are mepolizumab, and bertilimumab. Mepolizumab inhibits IL-5, an important mediator of eosinophil activation. The drug was prescribed to BP patients as adjuvant treatment to oral corticosteroids in a placebo controlled, double blind phase 2 pilot study.[61] While eosinophils were effectively reduced in skin and blood, mepolizumab showed no additive therapeutic value. Bertilimumab targets eotaxin-1, a potent chemoattractant and regulator of eosinophilic activity. No publications on bertilimumab for BP exist yet, however, ongoing studies are promising, and the drug was designated as orphan drug by the United States Food and Drug Administration for the treatment of BP.[100] A last drug of interest is apremilast, an phosphodiesterase 4 (PDE4) inhibitor registered for treatment of psoriasis. PDE4 inhibition reduced blistering in an experimental EBA mouse model, and our Center for Blistering Diseases currently performs a pilot study to examine the effectiveness and safety of apremilast in pemphigoid.[101]

Over the coming years, novel drugs need to demonstrate whether they have potential as therapeutic option for pemphigoid diseases. The frailty of the elderly pemphigoid population makes it challenging to include large numbers of patients in clinical trials, and emphasizes the need for multicenter collaborations. Beside the necessity for more therapeutic options it would be of great value to patientcare if future research could identify factors that can predict which treatment will be best suitable for individual patients. The ultimate goal for the future would be to realize a more personalized tailored disease management.

References

1. Schmidt T, Sitaru C, Amber K, Hertl M. BP180- and BP230-specific IgG autoantibodies in pruritic disorders of the elderly: a preclinical stage of bullous pemphigoid? *Br J Dermatol*. 2014;171(2):212-219. doi:10.1111/bjd.12936
2. Hofmann SC, Tamm K, Hertl M, Borradori L. Diagnostic value of an enzyme-linked immunosorbent assay using BP180 recombinant proteins in elderly patients with pruritic skin disorders. *Br J Dermatol*. 2003;149(4):910-912.
3. Chan LS, Vanderlugt CJ, Hashimoto T, et al. Epitope spreading: lessons from autoimmune skin diseases. *J Invest Dermatol*. 1998;110(2):103-109. doi:10.1046/j.1523-1747.1998.00107.x
4. Di Zenzo G, Thoma-Uszynski S, Calabresi V, et al. Demonstration of epitope-spreading phenomena in bullous pemphigoid: results of a prospective multicenter study. *J Invest Dermatol*. 2011;131(11):2271-2280. doi:10.1038/jid.2011.180
5. Cornaby C, Gibbons L, Mayhew V, Sloan CS, Welling A, Poole BD. B cell epitope spreading: mechanisms and contribution to autoimmune diseases. *Immunol Lett*. 2015;163(1):56-68. doi:10.1016/j.imlet.2014.11.001
6. Pawelec G. Age and immunity: What is "immunosenescence"? *Exp Gerontol*. 2018;105:4-9. doi:10.1016/j.exger.2017.10.024
7. van der Geest KSM, Abdulahad WH, Tete SM, et al. Aging disturbs the balance between effector and regulatory CD4+ T cells. *Exp Gerontol*. 2014;60:190-196. doi:10.1016/j.exger.2014.11.005
8. Antiga E, Quaglino P, Volpi W, et al. Regulatory T cells in skin lesions and blood of patients with bullous pemphigoid. *J Eur Acad Dermatol Venereol*. 2014;28(2):222-230. doi:10.1111/jdv.12091
9. Haeberle S, Wei X, Bieber K, et al. Regulatory T-cell deficiency leads to pathogenic bullous pemphigoid antigen 230 autoantibody and autoimmune bullous disease. *J Allergy Clin Immunol*. April 2018. doi:10.1016/j.jaci.2018.04.006
10. Muramatsu K, Ujiie H, Kobayashi I, et al. Regulatory T-cell dysfunction induces autoantibodies to bullous pemphigoid antigens in mice and human subjects. *J Allergy Clin Immunol*. 2018;142(6):1818-1830.e6. doi:10.1016/j.jaci.2018.03.014
11. Hashimoto T, Takahashi H, Sakaguchi S. Regulatory T-cell deficiency and autoimmune skin disease: Beyond the scurfy mouse and immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. *J Allergy Clin Immunol*. 2018;142(6):1754-1756. doi:10.1016/j.jaci.2018.08.028
12. Meijer JM, Diercks GFH, de Lang EWG, Pas HH, Jonkman MF. Assessment of Diagnostic Strategy for Early Recognition of Bullous and Nonbullous Variants of Pemphigoid. *JAMA dermatology*. 2019 Feb 1;155(2):158-165. doi:10.1001/jamadermatol.2018.4390
13. Sardy M, Kostaki D, Varga R, et al. Comparative study of direct and indirect immunofluorescence and of bullous pemphigoid 180 and 230 enzyme-linked immunosorbent assays for diagnosis of bullous pemphigoid. *J Am Acad Dermatol*. 2013;69(5):748-753. doi:https://dx.doi.org/10.1016/j.jaad.2013.07.009
14. Di Zenzo G, Della Torre R, Zambruno G, Borradori L. Bullous pemphigoid: from the clinic to the bench. *Clin Dermatol*. 2012;30(1):3-16. doi:10.1016/j.clindermatol.2011.03.005
15. Della Torre R, Combescure C, Cortés B, et al. Clinical presentation and diagnostic delay in bullous pemphigoid: A prospective nationwide cohort. *Br J Dermatol*. 2012;167(5):1111-1117. doi:10.1111/j.1365-2133.2012.11108.x

16. Hubner F, Recke A, Zillikens D, Linder R, Schmidt E. Prevalence and Age Distribution of Pemphigus and Pemphigoid Diseases in Germany. *J Invest Dermatol*. 2016;136(12):2495-2498. doi:10.1016/j.jid.2016.07.013
17. Fernandez-Viadero C, Arce Mateos F, Verduga Velez R, Crespo Santiago D. Blisters in a nursing home: bullous pemphigoid more often than we think? *J Am Geriatr Soc*. 2004;52(8):1405-1406. doi:10.1111/j.1532-5415.2004.52379_5.x
18. Cozzani E, Gasparini G, Burlando M, Drago F, Parodi A. Atypical presentations of bullous pemphigoid: Clinical and immunopathological aspects. *Autoimmun Rev*. 2015;14(5):438-445. doi:10.1016/j.autrev.2015.01.006
19. Försti AK, Jokelainen J, Ansakorpi H, et al. Psychiatric and neurological disorders are associated with bullous pemphigoid - A nationwide Finnish Care Register study. *Sci Rep*. 2016;6(September):1-6. doi:10.1038/srep37125
20. Milani-Nejad N, Zhang M, Kaffenberger J. The association between bullous pemphigoid and neurological disorders: a systematic review. *Eur J Dermatol*. 2017;27(5):472-481. doi:10.1684/ejd.2017.3066
21. Forsti A-K, Huilaja L, Schmidt E, Tasanen K. Neurological and psychiatric associations in bullous pemphigoid-more than skin deep? *Exp Dermatol*. 2017;26(12):1228-1234. doi:10.1111/exd.13401
22. Julio TA, Vernal S, Massaro JD, et al. Biological predictors shared by dementia and bullous pemphigoid patients point out a cross-antigenicity between BP180/BP230 brain and skin isoforms. *Immunol Res*. 2018;66(5):567-576. doi:10.1007/s12026-018-9028-1
23. Tuusa J, Lindgren O, Tertsunen H-M, et al. BP180 Autoantibodies Target Different Epitopes in Multiple Sclerosis or Alzheimer's Disease than in Bullous Pemphigoid. *J Invest Dermatol*. 2019;139(2):293-299. doi:10.1016/j.jid.2018.09.010
24. Kokkonen N, Herukka S-K, Huilaja L, et al. Increased Levels of the Bullous Pemphigoid BP180 Autoantibody Are Associated with More Severe Dementia in Alzheimer's Disease. *J Invest Dermatol*. 2017;137(1):71-76. doi:10.1016/j.jid.2016.09.010
25. Ben Mordehai Y, Faibish H, Astman N, Greenberger S, Barzilai A, Baum S. Characteristics of patients with bullous pemphigoid: comparison of classic bullous pemphigoid to non-bullous pemphigoid. *J Eur Acad Dermatol Venereol*. 2020 Jan;34(1):161-165. doi:10.1111/jdv.15883
26. Feliciani C, Joly P, Jonkman MF, et al. Management of bullous pemphigoid: the European Dermatology Forum consensus in collaboration with the European Academy of Dermatology and Venereology. *Br J Dermatol*. 2015;172(4):867-877. doi:10.1111/bjd.13717
27. Wang M, Lehman JS, Camilleri MJ, Drage LA, Wieland CN. Circulating bullous pemphigoid autoantibodies in the setting of negative direct immunofluorescence findings for bullous pemphigoid: A single-center retrospective review. *J Am Acad Dermatol*. 2019;81(2):472-479. doi:10.1016/j.jaad.2019.03.062
28. Ameen M, Harman KE, Black MM. Pemphigoid nodularis associated with nifedipine. *Br J Dermatol*. 2000;142(3):575-577. doi:10.1046/j.1365-2133.2000.03389.x
29. Lo Schiavo A, Ruocco E, Brancaccio G, Caccavale S, Ruocco V, Wolf R. Bullous pemphigoid: etiology, pathogenesis, and inducing factors: facts and controversies. *Clin Dermatol*. 2013;31(4):391-399. doi:10.1016/j.clindermatol.2013.01.006
30. Schmidt E, Obe K, Brocker EB, Zillikens D. Serum levels of autoantibodies to BP180 correlate

with disease activity in patients with bullous pemphigoid. *Arch Dermatol*. 2000;136(2):174-178. doi:10.1001/archderm.136.2.174
31. Patsatsi A, Kyriakou A, Pavlitou-Tsiontsi A, Giannakou A, Sotiriadis D. Association of autoantibodies to BP180 with disease activity in Greek patients with bullous pemphigoid. *Clin Dev Immunol*. 2012;2012:854795. doi:10.1155/2012/854795
32. Daneshpazhooh M, Ghiasi M, Lajevardi V, et al. BPDAI and ABSIS correlate with serum anti-BP180 NC16A IgG but not with anti-BP230 IgG in patients with bullous pemphigoid. *Arch Dermatol Res*. 2018;310(3):255-259. doi:10.1007/s00403-018-1817-9
33. Dopp R, Schmidt E, Chimanovitch I, Leverkus M, Brocker EB, Zillikens D. IgG4 and IgE are the major immunoglobulins targeting the NC16A domain of BP180 in Bullous pemphigoid: serum levels of these immunoglobulins reflect disease activity. *J Am Acad Dermatol*. 2000;42(4):577-583.
34. Feldrihan V, Licarete E, Florea F, et al. IgG antibodies against immunodominant C-terminal epitopes of BP230 do not induce skin blistering in mice. *Hum Immunol*. 2014;75(4):354-363. doi:10.1016/j.humimm.2014.01.005
35. Ujiie H, Yoshimoto N, Natsuga K, et al. Immune Reaction to Type XVII Collagen Induces Intramolecular and Intermolecular Epitope Spreading in Experimental Bullous Pemphigoid Models. *Front Immunol*. 2019;10:1410. doi:10.3389/fimmu.2019.01410
36. Yoshida M, Hamada T, Amagai M, et al. Enzyme-linked immunosorbent assay using bacterial recombinant proteins of human BP230 as a diagnostic tool for bullous pemphigoid. *J Dermatol Sci*. 2006;41(1):21-30. doi:10.1016/j.jdermsci.2005.11.002
37. Hall RP 3rd, Murray JC, McCord MM, Rico MJ, Streilein RD. Rabbits immunized with a peptide encoded for by the 230-kD bullous pemphigoid antigen cDNA develop an enhanced inflammatory response to UVB irradiation: a potential animal model for bullous pemphigoid. *J Invest Dermatol*. 1993;101(1):9-14.
38. Kiss M, Husz S, Janossy T, et al. Experimental bullous pemphigoid generated in mice with an antigenic epitope of the human hemidesmosomal protein BP230. *J Autoimmun*. 2005;24(1):1-10. doi:10.1016/j.jaut.2004.09.007
39. Groves RW, Liu L, Dopping-Hepenstal PJ, et al. A homozygous nonsense mutation within the dystonin gene coding for the coiled-coil domain of the epithelial isoform of BPAG1 underlies a new subtype of autosomal recessive epidermolysis bullosa simplex. *J Invest Dermatol*. 2010;130(6):1551-1557. doi:10.1038/jid.2010.19
40. He Y, Leppert J, Steinke H, Has C. Homozygous Nonsense Mutation and Additional Deletion of an Amino Acid in BPAG1e Causing Mild Localized Epidermolysis Bullosa Simplex. *Acta Derm Venereol*. 2017;97(5):657-659. doi:10.2340/00015555-2618
41. Turcan I, Pasmooij AMG, Gostynski A, et al. Epidermolysis Bullosa Simplex Caused by Distal Truncation of BPAG1-e: An Intermediate Generalized Phenotype with Prurigo Papules. *J Invest Dermatol*. 2017;137(10):2227-2230. doi:10.1016/j.jid.2017.04.041
42. Tanaka M, Hashimoto T, Dykes PJ, Nishikawa T. Clinical manifestations in 100 Japanese bullous pemphigoid cases in relation to autoantigen profiles. *Clin Exp Dermatol*. 1996;21(1):23-27.
43. Hayakawa T, Teye K, Hachiya T, et al. Clinical and immunological profiles of anti-BP230-type bullous pemphigoid: Restriction of epitopes to the C-terminal domain of BP230, shown by novel ELISAs of BP230-domain specific recombinant proteins. *Eur J Dermatol*. 2016;26(2):155-163.

doi:10.1684/ejd.2015.2719
44. Dainichi T, Chow Z, Kabashima K. IgG4, complement, and the mechanisms of blister formation in pemphigus and bullous pemphigoid. *J Dermatol Sci*. 2017;88(3):265-270. doi:10.1016/j.jdermsci.2017.07.012
45. Edwards G, Diercks GFH, Seelen MAJ, Horvath B, van Doorn MBA, Damman J. Complement Activation in Autoimmune Bullous Dermatoses: A Comprehensive Review. *Front Immunol*. 2019;10:1477. doi:10.3389/fimmu.2019.01477
46. Sadik CD, Miyabe Y, Sezin T, Luster AD. The critical role of C5a as an initiator of neutrophil-mediated autoimmune inflammation of the joint and skin. *Semin Immunol*. 2018;37:21-29. doi:10.1016/j.smim.2018.03.002
47. Romeijn TR, Jonkman MF, Knoppers C, Pas HH, Diercks GFH. Complement in bullous pemphigoid: results from a large observational study. *Br J Dermatol*. 2017;176(2):517-519. doi:10.1111/bjd.14822
48. Tao MH, Smith RI, Morrison SL. Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation. *J Exp Med*. 1993;178(2):661-667. doi:10.1084/jem.178.2.661
49. Jankaskova J, Horvath ON, Varga R, et al. Increased sensitivity and high specificity of indirect immunofluorescence in detecting IgG subclasses for diagnosis of bullous pemphigoid. *Clin Exp Dermatol*. 2018;43(3):248-253. doi:10.1111/ced.13371
50. Bernard P, Aucouturier P, Denis F, Bonnetblanc JM. Immunoblot analysis of IgG subclasses of circulating antibodies in bullous pemphigoid. *Clin Immunol Immunopathol*. 1990;54(3):484-494.
51. Al-Karawi KS. Immunoglobulin G subclass distribution of bullous pemphigoid autoantibodies and complement fixation studies. *Saudi Med J*. 2002;23(12):1492-1495.
52. Lamb PM, Patton T, Deng JS. The predominance of IgG4 in prodromal bullous pemphigoid. *Int J Dermatol*. 2008;47(2):150-153. doi:10.1111/j.1365-4632.2008.03361.x
53. Zheng M, Ujiie H, Iwata H, et al. Characteristics of IgG subclasses and complement deposition in BP230-type bullous pemphigoid. *J Eur Acad Dermatol Venereol*. 2019 Mar;33(3):595-600. doi:10.1111/jdv.15325
54. Buschman KE, Seraly M, Thong HY, Deng J-S, Draviam RP, Abernethy JL. A predominant IgG4 subclass may be responsible for false-negative direct immunofluorescence in bullous pemphigoid. *J Cutan Pathol*. 2002;29(5):282-286.
55. Budinger L, Borradori L, Yee C, et al. Identification and characterization of autoreactive T cell responses to bullous pemphigoid antigen 2 in patients and healthy controls. *J Clin Invest*. 1998;102(12):2082-2089. doi:10.1172/JCI3335
56. Echigo T, Hasegawa M, Shimada Y, Inaoki M, Takehara K, Sato S. Both Th1 and Th2 chemokines are elevated in sera of patients with autoimmune blistering diseases. *Arch Dermatol Res*. 2006;298(1):38-45. doi:10.1007/s00403-006-0661-5
57. Eming R, Budinger L, Riechers R, et al. Frequency analysis of autoreactive T-helper 1 and 2 cells in bullous pemphigoid and pemphigus vulgaris by enzyme-linked immunospot assay. *Br J Dermatol*. 2000;143(6):1279-1282.
58. Arakawa M, Dainichi T, Ishii N, et al. Lesional Th17 cells and regulatory T cells in bullous pemphigoid. *Exp Dermatol*. 2011;20(12):1022-1024. doi:10.1111/j.1600-0625.2011.01378.x
59. de Graauw E, Sitaru C, Horn M, et al. Evidence for a role of eosinophils in blister formation in

bullous pemphigoid. *Allergy Eur J Allergy Clin Immunol*. 2017;72(7):1105-1113. doi:10.1111/all.13131
60. van Beek N, Schulze FS, Zillikens D, Schmidt E. IgE-mediated mechanisms in bullous pemphigoid and other autoimmune bullous diseases. *Expert Rev Clin Immunol*. 2016;12(3):267-277. doi:10.1586/1744666X.2016.1123092
61. Simon D, Yousefi S, Cazzaniga S, et al. Mepolizumab failed to affect bullous pemphigoid: a randomized, placebo-controlled, double-blind phase 2 pilot study. *Allergy*. 2019 Jun 22. doi: 10.1111/all.13950. [Epub ahead of print]
62. Fairley JA, Baum CL, Brandt DS, Messingham KAN. Pathogenicity of IgE in autoimmunity: successful treatment of bullous pemphigoid with omalizumab. *J Allergy Clin Immunol*. 2009;123(3):704-705. doi:10.1016/j.jaci.2008.11.035
63. Kremer N, Snast I, Cohen ES, et al. Rituximab and Omalizumab for the Treatment of Bullous Pemphigoid: A Systematic Review of the Literature. *Am J Clin Dermatol*. 2019 Apr;20(2):209-216. doi:10.1007/s40257-018-0401-6
64. Kridin K. Peripheral eosinophilia in bullous pemphigoid: Prevalence and influence on the clinical manifestation. *Br J Dermatol*. 2018:1-7. doi:10.1111/bjd.16679
65. Hodge B, Roach J, Reserva JL, et al. The Spectrum of Histopathologic Findings in Pemphigoid: Avoiding Diagnostic Pitfalls. *J Cutan Pathol*. 2018;(August):1-8. doi:10.1111/cup.13343
66. Kamata A, Kurihara Y, Funakoshi T, et al. Basement membrane zone IgE deposition is associated with bullous pemphigoid disease severity and treatment results. *Br J Dermatol*. 2019 Jul 22. doi: 10.1111/bjd.18364. [Epub ahead of print]
67. Dimson OG, Giudice GJ, Fu CL, et al. Identification of a potential effector function for IgE autoantibodies in the organ-specific autoimmune disease bullous pemphigoid. *J Invest Dermatol*. 2003;120(5):784-788. doi:10.1046/j.1523-1747.2003.12146.x
68. Freire PC, Munoz CH, Stingl G. IgE autoreactivity in bullous pemphigoid: eosinophils and mast cells as major targets of pathogenic immune reactants. *Br J Dermatol*. 2017;177(6):1644-1653. doi:10.1111/bjd.15924
69. Moriuchi R, Nishie W, Ujiie H, Natsuga K, Shimizu H. In vivo analysis of IgE autoantibodies in bullous pemphigoid: a study of 100 cases. *J Dermatol Sci*. 2015;78(1):21-25. doi:10.1016/j.jdermsci.2015.01.013
70. Provost TT, Tomasi TBJ. Immunopathology of bullous pemphigoid. Basement membrane deposition of IgE, alternate pathway components and fibrin. *Clin Exp Immunol*. 1974;18(2):193-200.
71. Yayli S, Pelivani N, Beltraminelli H, et al. Detection of linear IgE deposits in bullous pemphigoid and mucous membrane pemphigoid: a useful clue for diagnosis. *Br J Dermatol*. 2011;165(5):1133-1137. doi:https://dx.doi.org/10.1111/j.1365-2133.2011.10481.x
72. Lamb PM, Abell E, Tharp M, Frye R, Deng JS. Prodromal bullous pemphigoid. *Int J Dermatol*. 2006;45(3):209-214. doi:10.1111/j.1365-4632.2004.02457.x
73. Murrell DF, Daniel BS, Joly P, et al. Definitions and outcome measures for bullous pemphigoid: recommendations by an international panel of experts. *J Am Acad Dermatol*. 2012;66(3):479-485. doi:10.1016/j.jaad.2011.06.032
74. Delaumenie S, Assikar S, Prudhomme R, et al. Methotrexate is safe and efficient as long-term treatment for bullous pemphigoid. *Eur J Dermatol*. 2019;29(2):217-218.

doi:10.1684/ejd.2019.3501
75. Kridin K, Bergman R. Mortality in Patients with Bullous Pemphigoid: A Retrospective Cohort Study, Systematic Review and Meta-analysis. *Acta Derm Venereol*. 2019 Jan 1;99(1):72-77. doi:10.2340/00015555-2930
76. Kalinska-Bienias A, Lukowska-Smorawska K, Jagielski P, Kowalewski C, Wozniak K. Mortality in bullous pemphigoid and prognostic factors in 1st and 3rd year of follow-up in specialized centre in Poland. *Arch Dermatol Res*. 2017 Nov;309(9):709-719. doi:10.1007/s00403-017-1772-x
77. Joly P, Baricault S, Sparsa A, et al. Incidence and mortality of bullous pemphigoid in France. *J Invest Dermatol*. 2012;132(8):1998-2004. doi:10.1038/jid.2012.35
78. Weisshaar E, Szepietowski JC, Dalgard FJ, et al. European S2k Guideline on Chronic Pruritus. *Acta Derm Venereol*. 2019;99(5):469-506. doi:10.2340/00015555-3164
79. Millington GWM, Collins A, Lovell CR, et al. British Association of Dermatologists' guidelines for the investigation and management of generalized pruritus in adults without an underlying dermatosis, 2018. *Br J Dermatol*. 2018;178(1):34-60. doi:10.1111/bjd.16117
80. Crowe S, Fenton M, Hall M, Cowan K, Chalmers I. Patients', clinicians' and the research communities' priorities for treatment research: there is an important mismatch. *Res Involv Engagem*. 2015;1:2. doi:10.1186/s40900-015-0003-x
81. Tallon D, Chard J, Dieppe P. Relation between agendas of the research community and the research consumer. *Lancet (London, England)*. 2000;355(9220):2037-2040. doi:10.1016/S0140-6736(00)02351-5
82. NETSCC, Cowan K. *The James Lind Alliance Guidebook 2006*. http://www.jla.nihr.ac.uk/jla-guidebook/downloads/JLA-Guidebook-Version-6-February-2016.pdf.
83. Polansky M, Eisenstadt R, DeGrazia T, Zhao X, Liu Y, Feldman R. Rituximab therapy in patients with bullous pemphigoid: A retrospective study of 20 patients. *J Am Acad Dermatol*. 2019;81(1):179-186. doi:10.1016/j.jaad.2019.03.049
84. Nicolas B, Pascal J, Marie-Laure G, et al. B-cell depletion induces a shift in self antigen specific B-cell repertoire and cytokine pattern in patients with bullous pemphigoid. *Sci Rep*. 2019;9(1):3525. doi:10.1038/s41598-019-40203-7
85. Maley A, Warren M, Haberman I, Swerlick R, Kharod-Dholakia B, Feldman R. Rituximab combined with conventional therapy versus conventional therapy alone for the treatment of mucous membrane pemphigoid (MMP). *J Am Acad Dermatol*. 2016;74(5):835-840. doi:10.1016/j.jaad.2016.01.020
86. Cho YT, Chu CY, Wang LF. First-line combination therapy with rituximab and corticosteroids provides a high complete remission rate in moderate-to-severe bullous pemphigoid. *Br J Dermatol*. 2015;173(1):302-304. doi:10.1111/bjd.13633
87. Green H, Paul M, Vidal L, Leibovici L. Prophylaxis of Pneumocystis pneumonia in immunocompromised non-HIV-infected patients: systematic review and meta-analysis of randomized controlled trials. *Mayo Clin Proc*. 2007;82(9):1052-1059. doi:10.4065/82.9.1052
88. Rekhtman S, Strunk A, Garg A. Incidence of pneumocystosis among patients exposed to immunosuppression. *J Am Acad Dermatol*. 2019;80(6):1602-1607. doi:10.1016/j.jaad.2018.12.052
89. Kowalski EH, Kneiber D, Kridin K, Amber KT. Comment on "Incidence of pneumocystosis among patients exposed to immunosuppression". *J Am Acad Dermatol*. 2019;81(2):e47.

doi:10.1016/j.jaad.2019.02.071
90. Rekhtman S, Strunk A, Garg A. Reply to: "Comment on 'Incidence of pneumocystosis among patients exposed to immunosuppression'". *J Am Acad Dermatol*. 2019;81(2):e49-e50. doi:10.1016/j.jaad.2019.03.072
91. Siscos SM, Neill BC, Tarantino IS, Aires DJ, Rajpara A. Dapsone advantages over trimethoprim-sulfamethoxazole for Pneumocystis pneumonia prophylaxis in immunobullous patients. *J Am Acad Dermatol* 2019 Feb 27. pii: S0190-9622(19)30346-9. doi:10.1016/j.jaad.2019.01.091
92. Kneiber D, Kowalski EH, Amber KT. Dapsone, two birds with one stone: a response to "Dapsone advantages over trimethoprim-sulfamethoxazole for Pneumocystis pneumonia prophylaxis in immunobullous patients". *J Am Acad Dermatol*. 2019 Apr 19. pii: S0190-9622(19)30631-0. doi:10.1016/j.jaad.2019.03.093
93. Joly P, Maho-Vaillant M, Prost-Squarcioni C, et al. First-line rituximab combined with short-term prednisone versus prednisone alone for the treatment of pemphigus (Ritux 3): a prospective, multicentre, parallel-group, open-label randomised trial. *Lancet (London, England)*. 2017;389(10083):2031-2040. doi:10.1016/S0140-6736(17)30070-3
94. Izumi K, Bieber K, Ludwig RJ. Current Clinical Trials in Pemphigus and Pemphigoid. *Front Immunol*. 2019;10:978. doi:10.3389/fimmu.2019.00978
95. Ellebrecht CT, Bhoj VG, Nace A, et al. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. *Science*. 2016;353(6295):179-184. doi:10.1126/science.aaf6756
96. Yayli S, Pelivani N, Beltraminelli H, et al. Detection of linear IgE deposits in bullous pemphigoid and mucous membrane pemphigoid: A useful clue for diagnosis. *Br J Dermatol*. 2011;165(5):1133-1137. doi:https://dx.doi.org/10.1111/j.1365-2133.2011.10481.x
97. Corti L, Fanoni D, Venegoni L, Muratori S, Recalcati S, Berti E. Detection of IgE autoantibodies in mucous membrane pemphigoid and their association with disease severity. *G Ital Dermatol Venereol*. 2018 Oct 4. doi:10.23736/S0392-0488.18.06167-9. [Epub ahead of print]
98. Koga H, Teye K, Yamashita K, Ishii N, Tsuruta D, Nakama T. Detection of anti-type VII collagen IgE antibodies in epidermolysis bullosa acquisita. *Br J Dermatol*. 2019;180(5):1107-1113. doi:10.1111/bjd.17310
99. Oya K, Watanabe R, Konishi R, et al. Possible involvement of IgE antibody in epidermolysis bullosa acquisita: detection and correlation. *Eur J Dermatol*. 2019;29(2):210-212. doi:10.1684/ejd.2019.3497
100. Kridin K, Ahn C, Huang WC, Ansari A, Sami N. Treatment Update of Autoimmune Blistering Diseases. *Dermatol Clin*. 2019;37(2):215-228. doi:10.1016/j.det.2018.12.003
101. Koga H, Recke A, Vidarsson G, et al. PDE4 Inhibition as Potential Treatment of Epidermolysis Bullosa Acquisita. *J Invest Dermatol*. 2016;136(11):2211-2220. doi:10.1016/j.jid.2016.06.619