Temporal outcomes after rituximab therapy for pemphigus vulgaris

Napatra Tovanabutra, M.D., Christina E. Bax, M.D., Rui Feng, Ph.D., Carolyn J. Kushner, M.D., Aimee S. Payne, M.D., Ph.D.

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Temporal outcomes after rituximab therapy for pemphigus vulgaris

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<th>PPV (95% CI)</th>
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Average DSG3 ELISA index value (RU/mL) at any point between months 3-9

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<td>0</td>
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Conclusion:
- Maximal prevalence of complete remission off oral systemic therapy (CROT) after one or more cycles of rituximab was 43.1%.
- DSG3 ELISA ≤130 RU/mL and ≥90% reduction at 3-9 mos, or DSG3 ELISA <20 RU/mL at 6-9 mos, may serve as a biomarker for future CROT.
- This dataset of temporal clinical and serologic outcomes will facilitate clinical trial planning and guide patient and physician expectations after rituximab therapy of pemphigus vulgaris.

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Authors: Napatra Tovanabutra M.D.1,2 (0000-0002-9463-5976), Christina E. Bax M.D.1 (0000-0003-1319-3318), Rui Feng, Ph.D.3 (0000-0003-4151-7228), Carolyn J. Kushne M.D.4 (0000-0002-7958-3186), Aimee S. Payne M.D., Ph.D.1 (0000-0001-9389-7918)

Authors’ affiliation:
1. Department of Dermatology, University of Pennsylvania, Philadelphia, USA
2. Department of Internal Medicine, Division of Dermatology, Chiang Mai University, Chiang Mai, Thailand
3. Department of Biostatistics, Epidemiology and Informatics, University of Pennsylvania, Philadelphia, USA
4. Department of Dermatology, New York University Langone Health, New York City, USA

Correspondence to:
Aimee S. Payne, MD, PhD
421 Curie Blvd
1009 Biomedical Research Building
Philadelphia, PA 19104
aimee.payne@pennmedicine.upenn.edu

Short title: Temporal outcomes after rituximab for pemphigus

Abbreviations: CR, complete remission; CROT, complete remission off oral systemic therapy; DSG3, desmoglein 3; mPV, mucosal-dominant pemphigus vulgaris; mcPV, mucocutaneous pemphigus vulgaris; PV, pemphigus vulgaris; RA, rheumatoid arthritis; ROC, receiver operating characteristic; RTX1, first rituximab cycle; RTX2, second rituximab cycle
Abstract

Pemphigus vulgaris is an autoimmune blistering disease characterized by autoantibodies that target desmoglein adhesion proteins. Rituximab and corticosteroids are FDA-approved therapies for pemphigus vulgaris. As newer treatments for pemphigus enter clinical trials, analysis of clinical and serologic outcomes after rituximab therapy as a function of time is essential to guide clinical trial design. Here, we report detailed temporal and serological outcomes of rituximab treatment of pemphigus vulgaris. The maximal prevalence of complete remission off oral systemic therapy after a single cycle of rituximab was 32.4% at 12 months, or 43.1% by 36 months including additional rituximab cycles. Using receiver operating characteristic curves to develop prediction models for complete remission after a single cycle of rituximab, >90.7% reduction in average desmoglein 3 ELISA titers from baseline to months 3-9 was 94% sensitive, and an average absolute titer ≤130 RU/mL between months 3-9 was 96% specific, for achievement of complete remission off oral systemic therapy. All patients with negative titers at 6-9 months ultimately achieved complete remission off oral systemic therapy. This dataset of clinical and serological outcomes for pemphigus vulgaris patients after rituximab therapy will facilitate clinical trial planning and also guide clinician and patient expectations after rituximab therapy.
Introduction

Pemphigus vulgaris (PV) is an autoimmune blistering disease characterized by autoantibodies targeting the desmosomal adhesion proteins desmoglein 3 (DSG3) +/- DSG1 (Kasperkiewicz et al., 2017). Rituximab and corticosteroids are first-line therapies for PV (Murrell et al., 2020) and are currently the only FDA-approved therapies for pemphigus. As newer PV treatments enter clinical trials, analysis of clinical and serologic outcomes after rituximab therapy for PV as a function of time is valuable to guide clinical trial design, including endpoint selection and timing, assessment timing, size, control group, and duration. Additionally, a better understanding of the temporal changes in DSG3 ELISA titer after rituximab therapy relative to clinical outcome would be valuable to evaluate its potential as a biomarker for complete disease remission off oral systemic therapy. Here, we report the temporal outcomes of rituximab treatment, including sub-analyses based on pemphigus vulgaris subtype and dosing regimen.

Results and Discussion

PV patients at the University of Pennsylvania were followed at least 12 months after first and second rituximab cycles (RTX1-2) (patient demographics, Table S1; eligibility, Figure S1). Outcomes followed international consensus definitions (Murrell et al., 2008). Primary clinical outcome, selected prior to case review, was complete remission off oral systemic therapy (CROT); composite secondary outcome was complete remission (CR), either on minimal therapy or CROT. Longitudinal anti-DSG3 ELISA titers were evaluated.

Clinical outcomes of rituximab therapy by time
Overall achievement of CROT. Prior studies have mostly reported cumulative probability of remission and relapse after rituximab (Cianchini et al., 2012, Heelan et al., 2014, Joly et al., 2017, Shimanovich et al., 2020, Vinay et al., 2018), which does not convey the dynamic nature of relapsed and refractory disease requiring re-treatment. We examined the overall distribution of clinical outcomes over 36 months in PV patients undergoing rituximab therapy, including rituximab re-treatment at the physician’s discretion for relapsed or refractory disease. Categories comprised 1) patients maintained in first CROT after a single cycle of rituximab (CROT1); 2) patients who required 2 or more cycles of rituximab for disease control due to previously refractory or relapsed disease (CROT2+); 3) patients with relapsed disease (almost all of whom received RTX2); and 4) refractory patients who had not achieved first CROT by the given timepoint despite one or more cycles of rituximab. The maximal prevalence of CROT1 was 32.4%, occurring 12 months after RTX1 (Figure 1). Additional rituximab cycles led to a 12-month CROT rate of 38.1% and maximal CROT prevalence of 43.1% at 36 months after RTX1 (Figure 1, green+yellow). These results are consistent with the 12-month CROT prevalence of 40.3% in a randomized controlled trial of rituximab in PV (Werth et al., 2021). CR prevalence peaked at 18 months (39.4% for CR1, 53.5% including CR2 or higher) (Figure S2).

Overall outcomes by RTX cycle, dose regimen, and PV subtype. Comparing outcomes from RTX1 and RTX2, overall CROT prevalence peaked at 12 months for both RTX1 (32.7%) and RTX2 (38.1%) (Figure S3a-b). The median time between RTX1 and RTX2 was 21.6 months (range, 6.5-56.9) in patients who previously achieved CROT and 12.3 months (range, 4.8-43.4) to improve response for those who did not achieve CROT after RTX1. Lymphoma-dose rituximab is associated with increased odds of achieving CROT compared to RA-dose, independent of patient body mass index (Kushner et al, 2019), presumably due to deeper B-cell
depletion in secondary lymphoid tissues. We identified a greater maximal prevalence of CROT with lymphoma- versus RA-dose, although differences decreased with subsequent rituximab cycles (38.5% versus 22.2% for RTX1, versus 42.5% and 34.8% for RTX2, respectively) (Figure S3a-b). Lymphoma-dosed patients demonstrated a longer median time to RTX2 of 16.6 months (95% CI 13.1-23.2), compared to 12.4 months with RA-dose (95% CI 8.46-18.8) (p = 0.037, log-rank test). There were comparable overall outcomes between mucocutaneous (mcPV) and mucosal-dominant (mPV) subtypes (Figure S3c-d). CR outcome patterns were similar (Figure S4a-d).

Cumulative probability of remission by RTX cycle, dose regimen, and PV subtype. To analyze the kinetics of remission and relapse contributing to the overall outcomes, we evaluated the cumulative probability of having achieved CROT/CR by a given timepoint, disregarding subsequent relapse (Figures S5a-b, S6a-b). Of 107 patients receiving RTX1, 51 (48%) achieved CROT1 and 63 (59%) achieved CR at any timepoint during 36-months follow up. Median time to CR was 13.2 months. 77 patients received RTX2, 63 of whom had complete medical records and follow-up greater than 12 months. 33 of these 63 patients (52%) achieved CROT a median of 21.5 months after RTX2, and 42 (67%) achieved CR after a median of 7.7 months. Figures S5c-f and S6c-f indicate cumulative probability of CROT/CR by dosing regimen and subtype, again revealing improved response with lymphoma-dose and comparable outcomes between PV subtypes.

Cumulative probability of relapse. We next examined the probability of relapse in patients who achieved CROT/CR after rituximab (Figure S7a-d). The cumulative probability of relapse was 61.5% and 51.5%, occurring a median of 18.3 months and 19.9 months after achieving CROT following RTX1 or RTX2, respectively. Similarly, 65.6% and 59.5% of
patients who achieved CR after RTX1 or RTX2 experienced disease relapse after a median duration of 18.3 months and 18.8 months. While significant differences were observed in time to CROT achievement (Figure S5a-b) with lymphoma- versus RA-dose, no notable differences were observed in rates of relapse after achieving CROT/CR based on dosing regimen (Figure S7a-d) or PV subtype. Similarly, while overall cumulative probability of CROT/CR as well as relapse after RTX2 is generally similar to RTX1, the median time to CROT/CR occurs earlier.

_Serologic outcomes of rituximab therapy of PV and relationship to clinical disease activity_

The mean duration of B-cell depletion after RA-dose rituximab is 8-9 months (Leandro et al., 2006, Roll et al., 2015, Thiel et al., 2017). The serum half-life of IgG in humans is approximately 3-5 weeks (Mankarious et al., 1988, Wasserman et al., 2009). Anti-DSG3 antibody titers fall below the ELISA cutoff value after rituximab therapy, indicating that the vast majority of autoantibodies are produced by short-lived plasma cells dependent on the CD20+ B-cell pool for replenishment (Joly et al, 2017). Disease relapse or incomplete remission after rituximab therapy of pemphigus is thought to be due to incomplete B-cell depletion, based on longitudinal B-cell repertoire cloning and spectratype analysis that shows recurrence or persistence of identical B-cell clones, although a role for long-lived plasma cells cannot be definitively ruled out (Colliou et al., 2013, Hammers et al., 2015). Thus, the anti-DSG antibody titer at a given point in time after rituximab therapy would be determined by the pharmacokinetics of B-cell depletion and repopulation, the serum IgG half-life, the baseline anti-DSG antibody titer, autoantibody affinity/avidity, and the depth of anti-DSG B-cell depletion by rituximab. To determine factors predictive of future achievement of CROT after rituximab, our study examined the absolute as well as percent change in DSG3 ELISA index values from
baseline through months 3-9 after a first cycle of rituximab therapy, with the expectation that a negative (<20 RU/mL) anti-DSG3 ELISA index value may be most likely to be observed 6-9 months after rituximab therapy, even in subjects with high baseline titers.

Blood samples were available at baseline prior to rituximab therapy and up to every 3 months after first rituximab infusion for some patients (129 serum samples from 44 patients). We evaluated DSG3 autoantibody titers after RTX1 (Figure 2a-b) using a linear mixed-effects model, adjusted for baseline values and within-subject correlation. There was no significant difference in baseline DSG3 titers between patients who ultimately achieved or did not achieve CROT (p=0.8285, 2-group t-test). CROT patients demonstrated greater average monthly reduction in DSG3 titers than non-CROT patients (p=1.2x10^-6). Using receiver operating characteristic (ROC) curves to develop prediction models for CROT, >90.7% reduction in DSG3 titers from baseline to months 3-9 was 94% sensitive, and an average absolute titer ≤130 RU/mL between months 3-9 was 96% specific, for achievement of future CROT (Figure 2c-d).

36 patients had blood samples available at baseline and months 6-9 after RTX1, a period of time when a negative DSG3 ELISA titer (<20 RU/mL) would be most likely based on the expected pharmacodynamic effects of rituximab and half-life of serum IgG. Notably, 12/12 patients with negative DSG3 ELISA titers (<20 RU/mL) at 6-9 months after RTX1 ultimately achieved CROT (Figure 3a), compared to 9/24 patients with DSG3 ELISA ≥20 RU/mL. More specifically, the DSG3 ELISA cutoff value of 20 RU/mL had 100% sensitivity for non-responsive disease, meaning that all 15 patients who ultimately did not achieve CROT demonstrated a DSG3 ELISA index value ≥20 RU/mL 6-9 months after RTX1. The DSG3 ELISA also had 100% negative predictive value for future achievement of CROT, indicating that among all 12 patients with a negative (<20 RU/mL) DSG3 ELISA index value between 6-9
months after RTX1, the probability of ultimately achieving CROT was 100%. Accurate estimation of the timing of the development of negative titers is affected by lack of serum samples at all timepoints. However, based on available samples, of the 12 patients who achieved CROT with negative DSG3 ELISA index values (<20 RU/mL) at 6-9 months after RTX1, 10 patients demonstrated their first negative titer a median of 88 (range, 11-217) days prior to CROT achievement. The remaining 2 patients demonstrated a DSG3 ELISA index value ≥20 RU/mL in the last assay prior to achievement of CROT and a negative titer in the first assay subsequent to achievement of CROT (68 or 99 days after CROT achievement), so it is unknown if development of negative titers occurred before or after CROT achievement.

6 of 9 patients (66.7%) who achieved CROT with DSG3 ELISA titers ≥20 RU/mL between months 6-9 after RTX1 experienced subsequent disease relapse, compared to 7 of 12 patients (58.3%) who achieved CROT with DSG3 ELISA titers <20 RU/mL between months 6-9. The median time to relapse among the 12 patients who achieved CROT with DSG3 ELISA <20 RU/mL was 34.2 months, compared to 11.3 months among the 9 patients who achieved CROT with DSG3 ELISA ≥20 RU/mL, although the difference was not significant (p=0.48 by log-rank test) (Figure 3b).

Assessment of DSG3 ELISA index values as a biomarker in pemphigus

In addition to autoantibody pharmacokinetics as discussed above, assessment of the relationship between DSG3 ELISA titers and clinical disease activity is affected by several factors. DSG3 ELISA titers have been shown to correlate with clinical disease activity when serum is serially diluted to within the linear range of the assay, whereas assays using a standard (1:101) serum dilution may fail to capture accurate changes in ELISA index value due to
saturation of antibody-antigen binding sites by high-titer sera (Cheng et al., 2002). Changes in manufacturing process for the commercial DSG3 ELISA (MBL International) in the mid-2000s resulted in incomplete propeptide cleavage, which disrupts DSG3 conformation and prevents binding of pathogenic but not non-pathogenic anti-DSG3 monoclonal antibodies (Sharma et al., 2009). Thus, MBL ELISA data obtained during this timeframe and/or assessments of high-titer sera without serial dilutions may be difficult to interpret. A second commercial ELISA (Euroimmun) with recombinant human DSG3 ectodomain produced without the propeptide to maximize the mature conformational protein was reported in 2010 (Schmidt et al., 2010). The Euroimmun assay uses a consistent assay cutoff value of 20 RU/mL, whereas the MBL International assay utilizes cutoffs for negative, indeterminate, and positive, whose absolute value may change between lots based on calibration against human serum control samples.

One of the largest prior studies on the correlation of pemphigus disease activity with DSG3 ELISA titer (424 serum samples from 80 patients using standard dilutions in an MBL assay) (Harman et al., 2001) showed a positive correlation between DSG1 antibodies and skin disease severity, as well as DSG3 antibodies and oral mucosal disease severity. A 10 U/mL increase in DSG1 ELISA index value was associated with 34% higher odds of higher skin severity scores, and a 10 U/mL increase in the DSG3 ELISA index value was associated with 25% higher odds of higher oral severity scores. No relationship was identified between DSG1 antibodies and oral disease severity, as expected given the predominant role of DSG3 in oral mucosal epithelial adhesion (Mahoney et al., 1999, Shirakata et al., 1998), or DSG3 antibodies and skin disease severity, potentially due to the lack of serial dilutions given the higher-titer sera used for correlation with skin versus oral disease activity, and/or lack of standardization in concomitant immunosuppressive medications (discussed further below).
A subsequent study examined 284 serum samples from 26 pemphigus patients (Abasq et al., 2009), including longitudinal samples from 20 participants in a prospective open-label study of rituximab (Joly et al., 2007). Mean DSG1 and DSG3 ELISA index values were lower during periods of complete remission on systemic immunosuppressive therapy compared to values during relapse, for both skin and mucosal disease. Although the conclusions of this study may be difficult to generalize given the timeframe of the study relative to manufacturing changes with the MBL ELISA and lack of serial serum dilutions, ROC curves identified a DSG1 ELISA index value of 20 U/mL (the assay negative cutoff value) as having 86% sensitivity, 78% specificity, 79% positive predictive value and 85% negative predictive value for the occurrence of skin relapses. The DSG3 ELISA cutoff value of 14 U/mL had 100% sensitivity but only 23% specificity for the occurrence of mucosal relapse. ROC curves indicated that a DSG3 ELISA cutoff value of 130 U/mL demonstrated 80% sensitivity, 84% specificity, 84% positive predictive value and 81% negative predictive value for the occurrence of mucosal relapses. Interestingly, our independent dataset, using serial serum dilutions and the Euroimmun ELISA, identified a DSG3 ELISA index value of 130 RU/mL between months 3 to 9 after rituximab therapy as the optimal cutoff value for predicting future achievement of CROT (Figure 2).

A prospective randomized clinical trial (Ritux3) of rituximab and short-term prednisone (n=46 subjects) versus high-dose prednisone alone (n=44 subjects) strongly supports a correlation between DSG3 ELISA value after treatment and achievement of CR off corticosteroids (Joly et al., 2017). Using serial serum dilutions and a Euroimmun assay, mean DSG3 ELISA titers fell below the negative cutoff value by day 720 in the rituximab/prednisone-treated but not high-dose prednisone-treated subjects, consistent with an 89% versus 34% rate of complete remission observed in these groups. Similarly, mean DSG3 titers were lower in the
rituximab/prednisone-treated subjects who remained in complete remission on maintenance dose rituximab compared to rituximab/prednisone-treated subjects who experienced disease relapse. A post-hoc analysis of the Ritux3 data (Mignard et al., 2020), using the same cutoff values identified in the prospective open-label study of rituximab in pemphigus (Abasq et al., 2009), identified that baseline Pemphigus Disease Area Index (PDAI) score of ≥45, plus DSG1 ELISA index value >20 RU/mL and/or DSG3 ELISA index value >130 RU/mL 3 months after rituximab treatment, had a positive predictive value of 50% and negative predictive value of 94% for the occurrence of early relapse. Based on these studies, the authors proposed that DSG3 ELISA titers 3 months after rituximab treatment might stratify patients who would most benefit from maintenance-dose rituximab at the 6-month timepoint to prevent disease relapse.

Another study used multivariate analysis to identify biomarkers for prediction of early relapse after rituximab in 62 pemphigus patients, including MBL DSG ELISA using standard serum dilutions (Albers et al., 2017). Positive DSG1 ELISA (≥20 U/mL) at any timepoint after therapy conferred a hazard ratio of 5.7 for relapse among patients with mucocutaneous disease, and positive DSG3 ELISA (≥20 U/mL) at any timepoint after therapy conferred a hazard ratio of 28.4 for relapse among patients with mucocutaneous and mucosal disease. Our study, using Euroimmun ELISA, did not find a significant difference between negative and positive DSG3 ELISA titers and median time to relapse (Figure 3b), although our analysis was restricted to months 6-9 after the first cycle of rituximab, whereas Albers et al. analyzed ELISA titers at any timepoint after therapy and included patients who received multiple rituximab cycles, which may be more likely to identify those at greater risk of relapse.

Collectively, these data support a strong correlation between disease activity and DSG ELISA titers, if properly assessed. However, a prospective observational study examining the
correlation of DSG ELISA values and disease activity scores in 116 pemphigus patients treated with standard care therapy (Hebert et al., 2019) questioned the strength of this association. At baseline, PDAI skin activity scores were highly correlated with DSG1 ELISA index values (r=0.84, p<0.0001) and PDAI mucosal activity scores were moderately correlated with DSG3 ELISA index values (r=0.62, p<0.0001), using serial serum dilutions and a Euroimmun assay. During the initial 3-month treatment period, the absolute improvement in PDAI skin activity scores was highly correlated with absolute decrease in DSG1 ELISA index value (r=0.75, p<0.0001), but weak correlation was observed between the absolute change in the PDAI mucosal activity scores and DSG3 ELISA index value (r=0.37, p<0.001). No correlation was observed between DSG ELISA index values and PDAI skin or mucosal scores during disease relapse. Of note, recent studies have shown that while DSG1 ELISA index values decrease during both rituximab and oral immunosuppressive therapy, DSG3 ELISA index values significantly decrease only after rituximab but not oral immunosuppressive therapies such as mycophenolate (Bhatia et al., 2020, Werth et al., 2021). Corticosteroids increase the transcription of DSG3 (Mao et al., 2017, Nguyen et al., 2004), which is thought to counteract the disruption of DSG3-mediated adhesion by pathogenic anti-DSG3 antibodies and facilitate the rapid healing of pemphigus lesions despite persistent circulating autoantibody titers. Thus, DSG ELISA titers, and particularly DSG3 ELISA titers, may best correlate with disease activity in the setting of B-cell depletion therapy and concomitant corticosteroid tapering regimens, given the potentially confounding effects of corticosteroids on disease activity.

Conclusions
In conclusion, this study provides a detailed dataset of clinical and serological outcomes for PV patients after rituximab therapy. The study is limited by its retrospective design, lack of quantitative assessments of disease activity, and unavailability of sera for all patients and timepoints. Reduction of DSG3 ELISA titers by >90.7% from baseline to months 3-9 or an average absolute titer of ≤130 RU/mL between months 3-9, and/or a negative DSG3 ELISA titer at months 6-9, may be valuable for predicting future CROT. A detailed understanding of temporal outcomes can facilitate clinical trial planning and guide clinician and patient expectations after rituximab therapy of pemphigus.
Materials and Methods

Patient Selection and Treatments

This study was performed under a protocol approved by the University of Pennsylvania Institutional Review Board. A waiver of written informed consent was granted for the retrospective chart review and use of sera previously collected for clinical purposes. The study population included all PV patients seen at the University of Pennsylvania from March 2005 until October 2017 and followed for at least 1 year after the first rituximab cycle. Subgroup analyses included a minimum follow-up of 1 year after both first and second rituximab cycles. Patients who required immunosuppressive agents for other diseases or had missing endpoint dates were excluded (Figure S1).

Diagnosis of PV was based on standard criteria (Murrell et al., 2008). A rheumatoid arthritis (RA) dose (1,000 mg on days 1 and 15) or lymphoma dose (375 mg/m2 weekly for 4 weeks) was administered based on physician discretion.

Outcome measurements

Clinical outcomes were determined using international consensus definitions (Murrell et al., 2008), including complete remission off oral systemic therapy (CROT) or complete remission (CR), a composite endpoint including either CROT or complete remission on minimal therapy. CROT is defined as the absence of new and/or established lesions while the patient is off all systemic therapy for at least two months. CR on minimal therapy is defined as the absence of new or established lesions while the patient is receiving less than or equal to 10 mg/day of prednisone (or the equivalent) and/or minimal adjuvant therapy for at least two months, with minimal adjuvant therapy being defined as half of the dose required to be defined as treatment.
failure (Murrell et al., 2008). Relapse is defined as the appearance of ≥3 new lesions/month that do not spontaneously heal within one week or the extension of established lesions.

Serologic outcomes were determined by ELISA. Patients with ≥2 available serum samples at baseline and at months 3 (range 1.5-4.5), 6 (range 4.5-7.5), 9 (range 7.5-10.5), or 12 (range 10.5-13.5) post first rituximab infusion were included (129 serum samples from 44 patients). Anti-DSG3 titers were determined using ELISA (Euroimmun, Lübeck, Germany) using serial dilutions of serum samples within the linear range of standard controls (Cheng et al., 2002). Corrected index values were calculated by multiplying index values by the dilution factor.

*Statistical analysis*

Baseline characteristics and clinical outcomes were summarized using descriptive statistics. Statistical analysis (R software, version 3.5.1) included time-to-event outcomes by Kaplan-Meier test and survival distributions by log-rank test. Statistical significance was defined by a p-value<0.05.

For analysis of serum autoantibody titers, DSG3 ELISA index values were natural log (ln)-transformed, replacing 0 values with 1 to make the transformation valid. A linear mixed effects model was used to assess change in ln-transformed ELISA index value between patients who achieved and did not achieve CROT, adjusted for baseline values and within-subject correlation. 95% confidence intervals for proportions are calculated according to the efficient-score method (corrected for continuity) (Newcombe, 1998).
Data Availability Statement
The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

ORCiD
Napatra Tovanabutra M.D. (0000-0002-9463-5976)
Christina E. Bax M.D. (0000-0003-1319-3318)
Rui Feng, Ph.D. (0000-0003-4151-7228)
Carolyn J. Kushner M.D. (0000-0002-7958-3186)
Aimee S. Payne M.D., Ph.D. (0000-0001-9389-7918)

Conflict of Interest Statement:
ASP: Cabaletta Bio equity, compensation, sponsored research, patent licensing; Novartis and Tmunity, patent licensing. NT, CEB, RF, and CJK report no conflicts of interest.

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Author Contributions:
Study conceptualization: ASP, NT; Data curation and analysis: NT, CEB, RF, CJK; Manuscript writing: ASP, NT, CEB; Manuscript review and editing: CEB, ASP, NT, RF, CJK.
References
Mao X, Cho MJT, Ellebrecht CT, Mukherjee EM, Payne AS. Stat3 regulates desmoglein 3 transcription in epithelial keratinocytes. JCI Insight 2017;2(9).


Figure Legends:

Figure 1. Overall achievement of CROT by time, including rituximab re-treatment. Graph indicates the overall percentage and number of patients who achieve and maintain CROT after a single cycle of rituximab (CROT1, green), achieve CROT after two or more cycles of rituximab (CROT2+, yellow), relapse after prior CROT (orange), and do not achieve CROT (Not CROT, gray). Patients were followed for up to 36 months after the first rituximab cycle and censored at end of follow-up; only patients with complete recording of dates through all RTX cycles were included (n=105).

Figure 2. Temporal changes in DSG3 autoantibody levels during the first cycle of rituximab therapy and future prediction of CROT. (a) Absolute and (b) percent change in DSG3 ELISA index values. (c) Receiver operating characteristic curve from CROT prediction models using an average DSG3 ELISA titer of 130 RU/mL between months 3-9 or 90.7% reduction in DSG3 ELISA titer from baseline to months 3-9, and (d) sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for future achievement of CROT after RTX1.

Figure 3. Relationship of negative versus positive DSG3 ELISA index values with future CROT achievement and disease relapse. (a) Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for future achievement of CROT after RTX1 with negative DSG3 ELISA index value (<20 RU/mL) at any timepoint between months 6-9. (b) Kaplan-Meier plot of time to disease relapse for patients who achieved CROT with DSG3 ELISA index values <20 RU/mL or ≥20 RU/mL between months 6-9 after RTX1. Vertical marks signify censored patients remaining in CROT at the indicated timepoints.

References


Mao X, Cho MJT, Ellebrecht CT, Mukherjee EM, Payne AS. Stat3 regulates desmoglein 3 transcription in epithelial keratinocytes. JCI Insight 2017;2(9).


Average percentage reduction in DSG3 ELISA index value at months 3-9

**80-100%**: 16, 8, 54% (71-100%), 67% (45-84%), 67% (45-84%), 54% (71-100%)

**>90.7%**: 1, 16, 54% (71-100%), 67% (45-84%), 67% (45-84%), 54% (71-100%)

Average DSG3 ELISA index values (IU/mL) between months 3-9

- **>130**: 9, 1, 53% (36-77%), 56% (73-99%), 94% (56-100%), 74% (65-81%)
- **≤130**: 23, 0, 23% (6-46%), 39% (26-85%), 91% (58-100%), 74% (65-81%)
a

<table>
<thead>
<tr>
<th>DSG3 ELISA Index value (RU/mL) at any point between months 6-9</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>&gt;=20</td>
</tr>
<tr>
<td>&lt;20</td>
</tr>
</tbody>
</table>

b

Survival analysis:
- Number at risk:
  - DSG3 ELISA <20 RU/mL
    - 12 at risk, 7 events
  - DSG3 ELISA >20 RU/mL
    - 9 at risk, 3 events

Event times:
- DSG3 ELISA >20 RU/mL
  - 1 event at 1 mos
  - 1 event at 3 mos
  - 1 event at 1 mos
  - 0 events at other times

Event times:
- DSG3 ELISA <20 RU/mL
  - 7 events at various times
a Time to CR after RTX1

b Time to CR after RTX2

c Time to CR after RTX1 by dosing regimen

d Time to CR after RTX2 by dosing regimen

e Time to CR after RTX1 by PV subtype

f Time to CR after RTX2 by PV subtype
### Table S1. Patient characteristics at the time of first and second rituximab therapy.

<table>
<thead>
<tr>
<th></th>
<th>First rituximab therapy (% total)</th>
<th>% Total</th>
<th>Second Rituximab therapy</th>
<th>% Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>107</td>
<td>100</td>
<td>63*</td>
<td>100</td>
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<tr>
<td>Gender</td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>48</td>
<td>45</td>
<td>26</td>
<td>41</td>
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<tr>
<td>Female</td>
<td>59</td>
<td>55</td>
<td>37</td>
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<td>Subtype</td>
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<tr>
<td>Mucocutaneous PV</td>
<td>87</td>
<td>81</td>
<td>53</td>
<td>84</td>
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<tr>
<td>Mucosal PV</td>
<td>20</td>
<td>19</td>
<td>10</td>
<td>16</td>
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<tr>
<td>Body mass index (BMI)</td>
<td></td>
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<tr>
<td>BMI median (range)</td>
<td>28.0 (16.6-52.5)</td>
<td></td>
<td>28.1 (18.6-53.2)</td>
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</tr>
<tr>
<td>Baseline antibody titer</td>
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<tr>
<td>Anti-DSG3** titers (RU/mL), median (range)</td>
<td>386.9 (25.0-1406.9)</td>
<td>148.4 (22.5-2255.1)</td>
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<td>Dose regimen</td>
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<tr>
<td>Lymphoma</td>
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<td>70</td>
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<td>Rheumatoid Arthritis</td>
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<td>Medication at rituximab treatment</td>
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<td>Prednisone</td>
<td>94</td>
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<td>≤10 mg/day</td>
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<td>23</td>
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<td>33</td>
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<td>11-20 mg/day</td>
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<td>21</td>
<td>9</td>
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<td>2000 mg/day</td>
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<td>16</td>
<td>9</td>
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<td>2500-3000 mg/day</td>
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<td>Azathioprine</td>
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<td>≤1.25 mg/kg/day</td>
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<tr>
<td>1.26-2.5 mg/kg/day</td>
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<td>IVIG*</td>
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<td>Other</td>
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</table>

*12 patients and 2 patients were excluded due to duration of follow-up <12 months after rituximab therapy or incomplete medical records, respectively.
**44 patients and 31 patients had available serum samples for first and second rituximab cycle evaluation, respectively.
*Intravenous immunoglobulin
Supplementary Legends

Figure S1. Patient eligibility. Flow diagram indicates criteria to determine patients included in the study analyses.

Figure S2. Overall achievement of CR. Graph shows overall achievement of CR, an accepted clinical outcome, when rituximab dosage and decision for re-treatment is based on physician discretion. Graph indicates the overall percentage and number of patients who achieve and maintain CR after a single cycle of rituximab (CR1, green), achieve CR after more than one cycle of rituximab (CR2 or higher, yellow), relapse after prior CR (orange), and do not achieve CR (gray). Only patients with complete recording of dates through all RTX cycles were included (n=105).

Figure S3. Achievement of CROT, subdivided by rituximab cycle, dosing regimen, and PV subtype. Graphs indicate the overall percentage and number of patients who achieve CROT (green), relapse after prior CROT (orange), and do not achieve CROT (gray) after the first and second rituximab cycles (RTX1 and RTX2). Panel (a) compares outcomes among PV patients overall (O) and those receiving lymphoma-dose (L) and Rheumatoid Arthritis-dose (RA) rituximab after RTX1 and Panel (b) after RTX2. Panel (c) compares outcomes among PV patients overall (O) and those with mucocutaneous or mucosal-dominant disease (mcPV and mPV) after RTX1 and Panel (d) after RTX2. Patients were followed for up to 36 months after the first rituximab cycle and censored at end of follow-up.

Figure S4. Achievement of CR, subdivided by rituximab cycle, dosing regimen, and PV subtype. Graphs indicate the overall percentage and number of patients who achieve CR (green), relapse after prior CR (orange), and do not achieve CR (gray) after the first and second rituximab cycles (RTX1 and RTX2). Panel (a) compares outcomes among PV patients overall (O) and those receiving lymphoma-dose (L) and Rheumatoid Arthritis-dose (RA) rituximab after RTX1 and Panel (b) after RTX2. Panel (c) compares outcomes among PV patients overall (O) and those with mucocutaneous or mucosal-dominant disease (mcPV and mPV) after RTX1 and Panel (d) after RTX2. Patients were followed for up to 36 months after the first rituximab cycle and censored at end of follow-up.

Figure S5. Cumulative probability of achieving CROT. Reverse Kaplan-Meier plot of time to CROT, disregarding potential subsequent disease relapse, for all PV patients (a) after RTX1 and (b) after RTX2, and divided by dose regimen (c-d) or PV subtype (e-f). No significant differences were observed in time to CROT based on PV subtype. p values calculated by log-rank test, statistical significance defined as p<0.05.

Figure S6. Cumulative probability of achieving CR. Reverse Kaplan-Meier plot of time to CR, disregarding potential subsequent disease relapse, for all PV patients (a) after RTX1 and (b) after RTX2, and divided by dose regimen (c-d) or PV subtype (e-f). p values calculated by log-rank test, statistical significance defined as p<0.05.

Figure S7. Cumulative probability of relapse-free survival after achieving CROT/CR. Kaplan-Meier plot of time to relapse after achieving CROT for all PV patients and separated by
dosing regimen after (a) RTX1 or (b) RTX2. Time to relapse after achieving CR after RTX1 and RTX2 appear in panels c-d. Vertical marks signify censored patients remaining in CROT/CR at the indicated timepoints.