# Appendices

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# English summary

This thesis presents studies that are focused on bullous pemphigoid (BP) and non-bullous pemphigoid (NBP). We investigated both diseases, their mechanisms and diseases connected with them, as well as the medication that can trigger them. In **Chapter 2** of the thesis we observed a statistically significant connection between BP and neurological disorders. BP180 and BP230 are expressed in the skin and the central nervous system, suggesting a potential link between neurodegeneration and BP. Multiple studies have also shown an increased risk of BP in patients with neurological diseases and vice-versa. Further investigations of this connection are necessary for a better understanding of both systems which will lead to better treatment. BP can be triggered by several medications and in **chapter 3** we described a wide range of pemphigoid subtypes observed in cancer patients, treated with immune checkpoint inhibitors (ICI). This diverse presentation included BP, NBP, mucous membrane pemphigoid, and Brunsting-Perry cicatricial pemphigoid. Pemphigoid needs to be considered as potential diagnosis in patients presenting with pruritic skin lesions during ICI therapy. Pruritus is commonly seen in BP and NBP and can severely affects patients' quality of life. **Chapter 4** focuses on pruritus and its treatment, highlighting that despite some recent discoveries, chronic pruritus remains poorly understood. This knowledge gap adversely affects patients and studies focusing on pruritus pathogenesis are crucial for effective treatments and better quality of life for affected individuals. IgE has a role in pruritus and is one of the key factors in allergies as well as autoimmune diseases, including BP. In **Chapter 5** of the thesis we observed elevated total IgE in the serum and skin of both NBP and BP patients, with pemphigoid-specific IgE more frequently detected in BP. This suggests shared IgE-dependent mechanisms in both diseases, particularly related to symptoms like pruritus. Anti-BP180 and BP230 IgE autoantibodies are associated with the severity of BP and omalizumab, an anti-IgE monoclonal antibody, has demonstrated efficacy in treating blisters and itching in BP patients. Our evolving understanding of IgE's role in pruritus and autoimmune diseases may pave the way for more precise and effective treatments across different medical conditions. Our understanding of the pathogenesis of blistering in BP remains incomplete. In our study described in **Chapter 6**, we found significantly higher numbers of both activated eosinophils and basophils in BP lesional skin compared to BP perilesional and NBP skin samples. This indicates their potential role in the inflammation and suggests also a role in the pathogenesis of the blistering. Further studies are needed to address if they also play a role in the blistering pathogenesis of BP. In **Chapter 7**, we explored the differences in the gene expression between BP and NBP in an effort to understand the differences in the pathogenesis. We discovered that genes related to complement

activation had higher expression in BP skin compared to NBP skin, suggesting a significant role in blister formation. Our study aligns with existing research, emphasizing complement's crucial role in BP blistering. Further investigations into gene expression in BP and non-BP are needed to expand on our preliminary findings.

# Nederlandse samenvatting

Dit proefschrift beschrijft studies gericht op bullous pemphigoïd (BP) en nonbullous pemphigoïd (NBP). We onderzochten beide ziekten, en dan met name ziekten die ermee in verband staan, medicatie die het kan uitlokken en onderliggende pathogenetische mechanismen.

In **hoofdstuk 2** van het proefschrift werd een statistisch significant verband tussen BP en neurologische aandoeningen waargenomen. BP180 en BP230 komen tot expressie in de huid en het centrale zenuwstelsel, wat een verband tussen neurodegeneratie en BP kan verklaren. Meerdere onderzoeken hebben naast ons onderzoek ook een verhoogd risico van BP aangetoond bij patiënten met neurologische aandoeningen en vice versa. Verder onderzoek naar dit verband is nodig, wat mogelijk zal leiden tot een betere behandeling van patiënten met zowel een neurologische aandoening als BP.

BP kan worden uitgelokt door verschillende medicijnen. In **hoofdstuk 3** beschreven we een breed scala aan subtypen van pemphigoïd bij kankerpatiënten die werden behandeld met immuuncheckpointremmers (ICI). Deze diverse presentatie omvatte BP, NBP, slijmvliespemfigoïd en Brunsting-Perry cicatricial pemphigoïd. Wij concluderen dat pemphigoïd als diagnose moet worden overwogen bij patiënten met jeukende huidlaesies tijdens ICI-therapie.

Pruritus komt vaak voor bij BP en NBP en kan de kwaliteit van leven van patiënten ernstig aantasten. **Hoofdstuk 4** richt zich op pruritus en de behandeling ervan en benadrukt dat ondanks enkele recente ontdekkingen, chronische pruritus nog steeds slecht begrepen wordt. Deze leemte in kennis heeft nadelige gevolgen voor patiënten. Studies die zich richten op de pathogenese van pruritus zijn cruciaal voor effectieve behandelingen en een betere levenskwaliteit voor patiënten met pemphigoïd.

IgE speelt een rol bij pruritus en is een van de belangrijkste factoren bij zowel allergieën als auto-immuunziekten, waaronder BP. In **hoofdstuk 5** observeerden we een verhoogd totaal IgE in het serum en de huid van zowel NBP- als BP-patiënten, waarbij pemphigoïd-specifiek IgE vaker werd gedetecteerd in BP. Dit suggereert gedeelde IgE-afhankelijke mechanismen in beide ziekten, met name gerelateerd aan symptomen zoals pruritus. Interessant is dat anti-BP180 en BP230 IgE autoantilichamen geassocieerd zijn met de ernst van BP. Ons voortschrijdend inzicht in de rol van IgE bij pruritus en auto-immuunziekten kan de weg vrijmaken voor nauwkeurigere en effectievere behandelingen bij pemphigoïd.

Ons begrip van de pathogenese van blaarvorming bij BP is nog onvolledig. In **hoofd-**

**stuk 6** beschrijven we significant hogere aantallen van zowel geactiveerde eosinofielen als basofielen in de lesionale huid van BP in vergelijking met perilesionale huidbiopten en NBP. Dit duidt op hun mogelijke rol in de ontsteking en suggereert ook een rol in de pathogenese van blaarvorming. Verdere studies zijn nodig om hun functionele rol in BP te onderzoeken.

In **hoofdstuk 7** werden de verschillen in genexpressie in huidbiopten tussen BP en NBP onderzocht. We ontdekten dat genen gerelateerd aan complementactivatie een hogere expressie hadden in BP vergeleken met NBP-huid, wat wijst op een belangrijke rol bij blaarvorming. Onze studie sluit aan bij bestaand onderzoek en benadrukt de cruciale rol van complement bij blaarvorming bij BP. Verder onderzoek naar genexpressies in BP en NBP is nodig om onze voorlopige bevindingen uit te breiden.

BP en NBP blijven zeldzaam, maar de incidentie neemt toe en met een beter begrip van de pathogenese is het mogelijk dat we betere en meer gerichte behandelingen kunnen ontwikkelen.

# Deutsche Zusammenfassung

In dieser Arbeit werden Studien vorgestellt, die sich auf das bullöse Pemphigoid (BP) und das nicht-bullöse Pemphigoid (NBP) konzentrieren. Diese beide Krankheitsbilder werden in Bezug auf ihre Mechanismen, und mögliche Folgeerkrankungen untersucht. Außerdem beleuchtet die Studie BP und NBP als Folge medikamentöser Behandlung. In Kapitel 2 der Arbeit wurde ein statistisch signifikanter Zusammenhang zwischen BP und neurologischen Störungen festgestellt. BP180 und BP230 werden in der Haut und im zentralen Nervensystem exprimiert, was auf einen möglichen Zusammenhang zwischen Neurodegeneration und BP hinweist. Mehrere Studien haben auch ein erhöhtes BP-Risiko bei Patienten mit neurologischen Erkrankungen und umgekehrt gezeigt. Um künftig bessere Behandlungsstrategien entwickeln zu können, ist ein tieferes Verständnis dieses Zusammenhangs notwendig.

BP kann durch verschiedene Medikamente ausgelöst werden. In **Kapitel 3** haben wir ein breites Spektrum von Pemphigoid-Subtypen, darunter BP, NBP, Schleimhautpemphigoid und Brunsting-Perry-Pemphigoid, analysiert. Diese wurden vor allem im Zusammenhang mit der Gabe von Immun-Checkpoint Inhibitoren (ICI) bei Krebspatienten beobachtet. Bei Patienten, die während einer ICI-Therapie juckende Hautläsionen aufweisen, muss ein Pemphigoid als Diagnose daher in Betracht gezogen werden.

Pruritus tritt häufig bei BP und NBP auf und kann die Lebensqualität der Patienten stark beeinträchtigen. **Kapitel 4** befasst sich mit Pruritus und seiner Behandlung und hebt hervor, dass der chronische Pruritus trotz einiger neuerer Entdeckungen nach wie vor nur unzureichend verstanden ist. Diese Wissenslücke wirkt sich nachteilig auf das Patientenwohl aus. Studien, die sich auf die Pathogenese des Pruritus konzentrieren, sind entscheidend für wirksame Behandlungen und eine bessere Lebensqualität der Betroffenen.

IgE spielt eine Rolle bei Pruritus und ist einer der Schlüsselfaktoren bei Allergien und Autoimmunerkrankungen, einschließlich BP. In **Kapitel 5** konnten wir erhöhte Gesamt-IgE-Konzentrationen im Serum sowie eine erhöhte Expression in der Haut sowohl von NBP- als auch von BP-Patienten feststellen. Dabei wurde pemphigoidspezifisches IgE häufiger bei BP nachgewiesen. Dies deutet auf gemeinsame IgE-abhängige Mechanismen bei beiden Krankheiten hin, insbesondere im Zusammenhang mit Symptomen wie Pruritus. Interessanterweise sind Anti-BP180- und BP230-IgE-Autoantikörper mit dem Schweregrad der BP korreliert. Unser wachsendes Verständnis der Rolle von IgE bei Juckreiz und Autoimmunerkrankungen könnte den Weg für präzisere und wirksamere Behandlungen bei verschiedenen

Erkrankungen ebnen. Unser Verständnis der Pathogenese der Blasenbildung bei BP ist aber noch unvollständig.

In **Kapitel 6** beschreiben wir eine signifikant höhere Anzahl von aktivierten Eosinophilen und Basophilen in der läsionalen Haut der BP im Vergleich zu perilesionalen und NBP-Hautproben. Dies weist auf ihre potenzielle Rolle bei der Entzündung hin und legt auch eine Rolle bei der Pathogenese der Blasenbildung nahe. Weitere Studien sind erforderlich, um ihre funktionelle Rolle bei BP zu entschlüsseln.

In **Kapitel 7** wurden die Unterschiede in der Genexpression zwischen BP und NBP untersucht. Gene, die mit der Komplementaktivierung zusammenhängen, werden in der BP-Haut stärker exprimiert als in der NBP-Haut; dies deutet auf eine wichtige Rolle bei der Blasenbildung hin. Unsere Studie deckt sich mit bestehenden Forschungsergebnissen, die die entscheidende Rolle von Komplement bei der Blasenbildung bei BP betonen. Weitere Untersuchungen der Genexpressionen bei BP und Nicht-BP sind erforderlich, um unsere vorläufigen Ergebnisse zu erweitern.

Obwohl BP und NBP weiterhin zu den seltenen Erkrankungen zählen, zeichnet sich eine Zunahme ihrer Häufigkeit ab. Mit einem vertieften Verständnis ihrer Pathogenese werden wir in der Lage sein, verbesserte und zielgerichtetere Behandlungsansätze zu entwickeln und gleichzeitig zu unserem Wissen über das Immunsystem beizutragen.

## Authors Independence declaration

I hereby declare that I have written this thesis titled “Bullous pemphigoid-What makes the blister?” by myself. I have written it independently and it has not been submitted for any other degree or professional qualification than the Joint Doctoral Degree of the University of Oldenburg and University of Groningen.

All of the used resources have been fully specified. I declare that the work submitted is my own work, done independently, unless the work was a part of a publication, done with other authors.

A list of publications has been included with this thesis and can be found on the next page. The research chapters (Chapter 2 to chapter 7), the author contributions have been written and all of the references have been made to the work of other authors. I am seeking the titles MD PhD (Doktorin) from the University of Oldenburg and PhD from the University of Groningen.

I also declare that this thesis is done according to the regulations on good academic practice of the University of Oldenburg. I declare enrolment as a doctoral candidate in accordance with Section 9 §5. No commercial services were used in connection with this doctoral project.

Nika Kotnik

# Publication list

Meyer, N. H., **Kotnik, N.**, Nzeteu, G. A. N., van Kempen, L. C., Mastik, M., Bockhorn, M., & Troja, A. (2024). Unraveling the MicroRNA tapestry: exploring the molecular dynamics of locoregional recurrent rectal cancer. Frontiers in Oncology, 14.

**Kotnik, N**., Langner, A., Meyer, N. H., Pas, H. H., Gibbs, B. F., Meijer, J. M., Diercks, G. F., Horváth, B & Raap, U. (2023). Infiltration analysis of eosinophils and basophils and co-expression of CD69, CD63, IL-31 and IgE in patients with bullous and non-bullous pemphigoid. Journal of the European Academy of Dermatology and Venereology.

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Franko, A., **Kotnik, N**., Goricar, K., Kovac, V., Dodic-Fikfak, M., & Dolzan, V. (2018). The influence of genetic variability on the risk of developing malignant mesothelioma. Radiology and oncology, 52(1), 105-111.

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I have started my PhD with a question-"What makes the blister?". While we don't have a definitive answer, I have, through my studies, helped shed a bit more light on the subject. I will keep an eye on the field of Immunology and BP, anticipating further discoveries.

All in all, I enjoyed my PhD. Was it hard sometimes? Yes? Were there moments when I doubted myself? Absolutely. But I had fun, I learned and I meet good and interesting people. I've gained valuable experiences and managed to work in two countries, do my PhD at two universities. And while I've spent way more time at that station in Leer than I thought I would, I'm happy to have been a part of this cross border cooperation. In the end, it was good. I have extremely fond memories of Oldenburg and Groningen and I hope to keep in contact with the people that I've met and worked with.

## About the author

Nika Kotnik, dr.med, the author of this thesis, was born in Kranj, (SLO) on the 5th of August, 1988. She finished primary school and high school in Kranj, and finished her high school final exams with the highest amount of points (34). She then studied Medicine at the University of Ljubljana (SLO), where she graduated in 2015. During her studies, she did research work in the field of genetics and received the faculty's award (Prešeren's award) for it. After an internship at the country's largest hospital in Ljubljana, she started her PhD work, titled "Bullous pemphigoid-what makes the blister?", under the mentorship of Prof. Dr. Ulrike Raap and the late Prof. Dr. Marcel Jonkman. She lived in Oldenburg for 2 years and a half (Feb 2018-Sept 2020) and worked at the group for Experimental Allergology and Immunodermatology. She then continued her work in Groningen (Sept 2020-Mar 2022), at UMCG with the Dermatology group. After the tragic death of Prof. Dr. Marcel Jonkman, Prof. Dr. Barbara Horvath took over as her PhD mentor.

After her PhD she will pursue a specialty in immunology and allergology in Slovenia, working as an MD. She plans to combine the knowledge and expertise she gained during her PhD with her medical background to work with patients as well as continuing her work in research.