PROGRAM ABSTRACTS NVED 2023

ORAL PRESENTATIONS

1 – Thamila Kerkour

Automated assessment of histological tissue structures by artificial intelligence to predict distant metastasis in cutaneous melanoma.

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Background Important prognostic histopathological features (e.g. mitosis) are currently not included in staging systems, due to discrepancies in scoring between pathologist and being time-consuming. Currently available melanoma algorithms are subject to a weak performance or feature specific detection.

Objective Development of automated assessment of multiple histological tissue structures of cutaneous melanoma (CM), which can be used in prognostic models.

Methods We trained convolutional neural networks for CM histological structures detection on whole slide image (WSI), using step-wise applications in Visiopharm. For the training cohort, 66 digital hematoxylin and eosin (H&E) slides of diagnostic excision of melanoma patients were used, magnification 40x. Annotations of the tumor area, epidermis, hair follicles, sebaceous glands and mitosis were made manually. To evaluate the performance of the applications, the automated segmentation outputs were validated on 10 slides. Performance measures consisted on Dice score (at least 85%, i.e. true positive tissue of all tissue detected by the app).

Results Five applications were developed for a step-wise automated detection of: the epidermis (1) based on DeepLab3+ architecture, tumor region (2), sebaceous gland (3), hair follicles (4) based on the U-Net architecture, and mitosis within the tumor area (5) based on DeepLab3+ architecture. All the applications achieved a Dice score >94% except for the tumor application with Dice score of 80 %, due to the presence of single tumor cells within the dermis and epidermis.

Conclusion These applications provide us valuable tools to segment relevant tissue structures which can be validated in prediction models for distant metastasis in cutaneous melanoma.

2 – Celeste Eggermont

Development and Validation of a Prediction Model for the Occurrence of Subsequent Cutaneous Squamous Cell Carcinoma

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Background One third of patients with cutaneous squamous cell carcinoma (cSCC) will develop subsequent cSCCs, but there is a lack of data to inform guidelines on the frequency of follow-up.
Objective To develop and validate a model that predicts the absolute risk of developing subsequent cSCCs.
Methods All patients with a first cSCC in 2007/2008 from the Netherlands Cancer Registry were included and linked to 1) the Netherlands Pathology Registry for data on subsequent cSCCs during a 10-year follow-up and 2) the Netherlands Organ Transplant Registry. Similar data was obtained for patients in 2009/2010 for external validation. Candidate predictors of the joint frailty models included time-fixed variables (age, sex) and time-varying variables (organ transplantation, hematological malignancy, topography and differentiation grade of the previous cSCC, number of previous cSCCs, and time-interval between previous cSCCs).
Results Among the 12,345 included patients from 2007/2008, second to sixth cSCCs occurred in 4,325, 2,010, 1,138, 739, and 501 patients, with median follow-up times of 1.4, 1.2, 0.9, 0.6, and 0.5 years after the previous cSCC, respectively. The cumulative incidence of developing a subsequent cSCC at 5 years was 27%, 42%, 54%, 64%, and 67% for the second to sixth cSCC, respectively. Separate models were developed for solid organ transplant recipients (SOTRs) and non-SOTRs. The number of previous cSCC diagnosis dates was found to be the strongest predictor for both groups.
Conclusion These absolute risk models allow for the identification of patients at high risk of a new cSCC and will facilitate individualized decision-making for follow-up.

3 – Tom Wolswijk

'Optical coherence tomography for diagnosing recurrent basal cell carcinoma after non-invasive treatment'

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Background Superficial basal cell carcinoma (sBCC) can be treated non-invasively, but follow-up is necessary because lesions can reoccur. However, recurrences may not always be visible during clinical and dermoscopic examination (CDE). Optical coherence tomography (OCT), a non-invasive diagnostic modality, may detect these subclinical recurrences.
Objective To compare the diagnostic accuracy of CDE and CDE with addition of OCT (CDE-OCT) for detection of recurrent BCC after non-invasive treatment of sBCC.
Methods In this diagnostic cohort study the treating physician performed CDE after which an OCT scan was obtained and assessed. The treating physician and OCT assessor recorded their suspicion level for BCC recurrence on a 5-point Likert-scale. Patients with high suspicion of BCC recurrence (positive test result) based on CDE and/or CDE-OCT underwent punch biopsy. Patients with a low suspicion (negative test result) on CDE and CDE-OCT were asked to (voluntarily) undergo a control biopsy. Histopathological results of biopsy specimens were used for verification of the CDE and CDE-OCT diagnoses (gold standard).
Results Included were 100 patients. Results will be discussed during the NVED Annual Meeting 2023.
Conclusion The conclusion of this study will be discussed during the NVED Annual Meeting 2023.

4 – Anna Zwanenburg

Clinical features and outcomes of advanced basal cell carcinoma

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Background Basal cell carcinoma (BCC) is a mostly indolent and easily curable tumour. However, locally advanced BCCs (laBCC) present a therapeutic challenge, and while metastatic behavior is rare, the prognosis is poor.

Objective To report the characteristics and clinical course of laBCC and metastatic BCC (mBCC).

Methods A retrospective cohort study was conducted at a tertiary oncological hospital over a 32 year period to identify all laBCCs and mBCCs. Patient-, tumour-, and clinical outcome characteristics were recorded.

Results Fifty-one patients were included, 23 mBCC and 28 laBCC, with a median follow-up of 22 months. Thirty-five patients were men (69%), with a mean age of 72 years. Most primary BCCs were located in the head-and-neck area (30, 59%), and were primarily treated with surgery (78%). Eleven laBCCs occurred in a recurrent tumour (39%). Giant BCC was seen in 18 laBCCs (64%) and a large subset was larger than 9cm. Of laBCC, 50% experienced local recurrence after resection, which was seen in 35% of mBCCs. Median time to metastasis was 33 months. Most patients developed nodal metastases only (70%), but 26% developed oligometastasis. Fifteen patients died during follow-up (29%), of which 7 of BCC (3 laBCC, 4 mBCC). Median overall survival (OS) was 49 months. Five year OS was 79% for laBCC and 83% for mBCC.

Conclusion Treatment of laBCC and mBCC consists mostly of surgery, but tumour recurrence was high in all advanced BCCs. Prognosis of these tumors is relatively poor, highlighting the need for better follow-up strategies and treatment options for these patients.

5 – Patrick Mulder

Full Skin Equivalents for Simulation of Burn Wound Healing and Inflammatory Response

Patrick P.G. Mulder[1,2], Lotte Rozemeijer[1], Leonore S. Mastenbroek[1], Marcel Vlig[1], Anouk Elgersma[1], Rajiv S. Raktoe[1], Esther Middelkoop[1,3,4], Irma Joosten[2], Hans J.P.M. Koenen[2], Bouke K.H.L. Boekema[1,3]

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Background Healing of burn injury is a complex process that often leads to functional and aesthetic complications. To study skin regeneration and inflammation in more detail we used full skin equivalents (FSEs) generated from dermal matrices.

Objective The aim of this study was to validate the MatriDerm®- and Mucomaix®-based FSEs for the use as in vitro models of burn wound healing. In addition, we studied the effect of burn injury on immune cells.

Methods Immunohistochemistry was used to study skin development, regeneration and cell proliferation in FSEs. T cells and monocytes from buffy coat were added to the Matriderm-based FSEs and changes in phenotype and cytokine profile caused by burn injury were studied using flow cytometry.

Results Proper dermal and epidermal morphogenesis was established in all FSEs. Organization of the epidermal layers and the basement membrane in MatriDerm-based FSE improved with time, while Mucomaix-based FSE rapidly degraded. Re-epithelization occurred in the DED- and MatriDerm-based FSEs at 2 weeks after injury, similar to ex vivo human skin. High levels of pro-inflammatory cytokines were produced by FSEs, which were increased in the presence of monocytes or T cells. Monocytes in FSEs differentiated towards CD68+ macrophages in 7 days, while burns increased their HL-DR expression. After burn injury and in the presence of eschar lysate more CD183+ T cells were observed.

Conclusion We anticipate that these animal-free in vitro models can facilitate research on skin regeneration and can be used to test therapeutic interventions in a preclinical setting to improve wound healing.

6 – Andrew Morrison

Developing a 3D human lymph node model to study inflammatory diseases

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Background Lymph nodes are secondary lymphoid organs that are fundamental in orchestrating adaptive immune responses. Their highly specialized architecture is regulated by non-hematopoietic Fibroblast Reticular Cells (FRCs), which support immune cell function, e.g. facilitating entry of Dendritic Cells (DCs) after exposure-induced migration from skin. The study of human antigen and tissue-specific adaptive immune responses arising from inflammatory stimuli requires competent in vitro models recapitulating the physiological microenvironment.

Objective To create a static 3D human lymph node model containing FRCs to study their interaction and influence on MUTZ-3, a cell line that can differentiate into DCs.

Methods Primary human FRCs were isolated from lymph node biopsies and co-cultured with either MUTZ-3 progenitors or MUTZ-3 DC in a 3D collagen-fibrin hydrogel. At the end of the culture period, cell profiles were characterized through flow cytometry and cytokine/chemokine analysis, as well as histology and 3D imaging to visualize cellular localisation.

Results The presence of FRCs in the hydrogel improved the viability of MUTZ-3 DC cells and were also found to influence the development of MUTZ-3 DCs from MUTZ-3 progenitors under inflammatory stimuli to a lymph node-resident DC-like phenotype. Imaging revealed direct cell to cell contact between FRCs and MUTZ-3 DCs.

Conclusion This model highlights the importance of FRCs in regulating DC behaviour in a lymph node model, proving useful for further in-depth studies of stroma-immune cell interactions emerging from allergens and inflammatory stimuli. Such a 3D platform presents a beneficial opportunity for implementation into microfluidic multi-organ-on-chip settings, such as a skin-draining lymph node.

7 – Jonas Jäger

Reconstructed Human Skin with Hypodermis Shows Essential Role of Adipose Tissue in Skin Metabolism

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Background Subcutaneous adipose tissue plays a role in wound healing, hair follicle cycling, a plethora of diseases and is metabolically very active. However, current reconstructed human skin (RhS) models do not incorporate an adipocyte containing hypodermis and exhibit low metabolic potential.
Objective To determine whether the incorporation of an adipocyte-containing hypodermis into RhS will improve its metabolic potential and to identify the major metabolic pathways involved.
Methods Primary human keratinocytes, fibroblasts and differentiated adipose-derived stromal cells (ASCs) were co-cultured in a collagen/fibrin scaffold to create an adipose-RhS. The model was characterized structurally in 2D and 3D, histology, cytokine secretion and metabolic enzyme expression (RNA-sequencing).
Results Lipid droplet formation, gene expression of key adipogenic markers and adipokine secretion confirmed differentiation of ASCs to adipocytes. Addition of the adipose layer resulted in up-regulation of 286 genes in the dermal-adipose layer of adipose-RhS compared to RhS without adipocytes. These genes were predominantly involved in vitamin A and vitamin D metabolic pathways. Out of the up-regulated genes, 7 were identified as phase I and II metabolic enzymes. Cytokine secretion profile showed reduced concentrations of pro-inflammatory cytokines in adipose-RhS.
Conclusion Adipose-RhS has a less inflamed phenotype indicating the contribution of adipocytes to tissue homeostasis. Up-regulated enzymes show a higher metabolic activity of the adipose-RhS. Taken together, our results show the essential role of adipocytes in the hypodermis enabling adipose-RhS to mimic native human skin more closely than RhS. This opens exciting new possibilities to investigate the human skin in health and disease in the future.

8 – Luca Meesters

A data-driven approach towards mature iPSC-derived keratinocytes

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Background Induced pluripotent stem cell (iPSC)-derived keratinocytes (iKCs) show great potential to model monogenetic skin diseases, yet generation of organotypic skin models to study inflammatory skin diseases has not been reported. iPSCs can be derived from patient cells or be genome edited, have an infinite lifespan and can differentiate towards various skin cell types. However, iKC survival, proliferation, cellular heterogeneity and immaturity hamper wide implementation in organotypic skin modeling.

Objective and methods To improve cell culture protocols by targeted and data-driven optimization strategies.

Results CELLnTEC (CnT)-30 medium greatly increased the iKC culture homogeneity as compared to keratinocyte serum free medium (KSFM). Transcriptomic analysis showed significantly higher basal keratinocyte marker expression (100-fold higher keratin 5) and lower fibroblast marker expression (10-fold lower vimentin), and in general a morphology and transcriptome more closely resembling that of primary keratinocytes. KEGG pathway analysis of differentially regulated genes indicated putative targets for improvement of CnT-30 by small molecule compounds, including inhibition of PI3K-Akt and JAK-STAT signaling. We combined this data-driven optimization approach with hypothesis-driven alterations in coating strategies (Geltrex + collagen I/IV) and seeding densities, showing marked differences in growth rate and differentiation status.

Conclusion iKC expansion appeared challenging, which would be crucial to overcome for future organotypic skin model development. We postulate that the iKC maturity required for becoming epidermal progenitor cells is key and requires further optimization of the culture medium composition. This will contribute to the future applicability of iPSCs as indefinite cell source for inflammatory skin disease modeling.

9 – Jasper Koning

Vascularization of multi-Organ-on-Chips with blood and lymphatic endothelial cells for the generation of immunocompetent skin models

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Background Until now, 3D human skin models fail to include both blood (BEC) and lymphatic endothelial cells (LEC) despite their essential role for homeostasis and immune responses, limiting their relevance for disease modeling and safety testing. Therefore, establishment of vascularized Organ-on-Chip microfluidic bioreactors with BECs and LECs are a pre-requisite to further improve human skin models to study human diseases.

Aim Set up a robust and reproducible method for the vascularization of organ-on-chip microfluidics with human BECs or LECs which allows long term culturing under physiologic flow conditions for future immunocompetent multi-Organ-on-Chip models.

Methods Human skin endothelial cells were separated into BECs and LECs, expanded, used to vascularize multi-Organ-on-Chips and cultured for up to 14 days under dynamic flow conditions mimicking blood and lymph flow pressures. Morphology, mRNA expression and biomarkers profiles in culture supernatants was investigated upon homeostatic and inflammatory conditions.

Results The new method results in large numbers of pure BECs and LECs. Upon vascularization and prolonged culture periods in Organ-on-Chips, cells retained their endothelial specific phenotype. Biomarker expression of BECs and LECs was different and the cells respond to inflammatory conditions by upregulating various biomarkers. mRNA levels of endothelial junction markers did not substantially change, while the LEC specific markers were reduced in LECs upon inflammatory conditions.

Conclusion The presented method can be used to further enhance organ-on-chip models through the incorporation of functional BECs and LECs resulting in relevant healthy and diseased tissue models to investigate human disease and safety testing.

10 – Charlotte Burms

Palmoplantar keratoderma as a clinical feature of pathogenic variants in the filaggrin gene

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Background Pathogenic variants in the filaggrin (FLG) gene cause ichthyosis vulgaris (IV) and predispose to atopic dermatitis. Hereditary palmoplantar keratoderma (PPK) can be an isolated condition or a part of another genodermatosis, such as ichthyosis. FLG variants are suggested modifiers of PPK. To our knowledge, pathogenic variants in the FLG gene have not been described before as a cause of PPK and PPK has not been included in the list of FLG-related symptoms.

Objective We investigated a hypothesis that variants in FLG could cause PPK.

Methods We screened databases of two expertise centers for genodermatoses and included22 patients with PPK, pathogenic variants in the FLG gene and no other genetic or clinical cause of PPK. Genetic testing was done with whole exome sequencing (18/22) and targeted analysis for FLG was performed (4/22). Phenotypical characteristics were retrieved from the patient files.

Results Ten patients were heterozygous (FLG -/+), six homozygous and six compound heterozygous for pathogenic FLG variants (FLG -/-). Two novel variants, c.10086del and c.5842G>T, were identified. All patients presented with diffuse PPK, generalized xerosis cutis and almost all (20/22) showed hyperlinearity on the palms of the hands. The palmoplantar skin changes did not fit the clinical presentation of eczema and itch was not reported.

Conclusion We show that pathogenic variants in the FLG gene can cause PPK as an IV-related symptom. We propose screening of the FLG gene in case of a diffuse PPK and adding PPK to the list of symptoms of IV.

11 – Hanna Niehues

CYSRT1: an antimicrobial epidermal protein that can interact with late cornified envelope (LCE) proteins

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Background Late cornified envelope (LCE) proteins are small cationic epidermal proteins with antimicrobial properties, and the combined deletion of LCE3B and LCE3C genes is a risk factor for psoriasis that affects skin microbiome composition.

Objective Identification of protein-protein interactions is a powerful way to understand protein function and contributes to our understanding of how dysregulation of these interactions can contribute to disease.

Methods We screened for binding partners of LCE proteins to determine interactions with skin-specific proteins that attribute to skin barrier function or host defense and studied expression patterns and protein function thereof.

Results In a yeast two-hybrid screen we identified cysteine-rich tail 1 protein (CYSRT1) as an interacting partner of members of all LCE groups except LCE6, and confirmed this with mammalian co-immunoprecipitation. CYSRT1 is a protein of unknown function, specifically expressed in cutaneous and oral epithelia and spatially colocalizes with LCE proteins in the upper layers of the suprabasal epidermis. Constitutive CYSRT1 expression is present in fully differentiated epidermis and can be further induced *in vivo* by disruption of the skin. Transcriptional regulation correlates to keratinocyte terminal differentiation but not to skin bacteria exposure. Similar to LCEs, CYSRT1 was found to have antibacterial activity against Pseudomonas aeruginosa. Comparative gene sequence analysis and protein amino acid alignment indicates that CYSRT1 is highly conserved among vertebrates and has putative antimicrobial activity.

Conclusion We here identified CYSRT1 in the stratum corneum skin layer, where it colocalizes with LCE proteins and contributes to the constitutive epidermal antimicrobial host defense repertoire.

12 – Vanya Rossel

Novel variants in Desmoglein 1 causing Severe skin dermatitis, multiple Allergies and Metabolic wasting (SAM) syndrome and palmoplantar keratoderma

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Background Biallelic loss-of-function variants in *DSG1* encoding desmoglein 1 cause severe atopic dermatitis, multiple allergies, and metabolic wasting (SAM) syndrome, whereas heterozygous variants cause palmoplantar keratoderma (PPK).

Objectives Investigating the genetic variations, pathophysiology and clinical findings of two patients with SAM syndrome and eleven patients with PPK.

Methods Patients were recruited from European medical centres. Genetic analysis was used to identify variants in *DSG1*. Immunofluorescence staining was performed to determine DSG1 protein expression and localization in SAM patients. Clinical data were extracted from patient records.

Results Here, we report two SAM patients and eleven PPK patients from unrelated families and identified novel variants in *DSG1*. In a severe and in a mild SAM patient, we identified compound heterozygous and homozygous variants, respectively. Consequently, the variants result in total or partial absence of DSG1 in the epidermis. Clinical heterogeneity in PPK patients was seen, with striate, focal or a diffuse PPK.

Conclusion This study demonstrates the genetic and clinical heterogeneity in SAM and PPK due to *DSG1* variants and adds novel pathogenic *DSG1* variants, including rare missense variants. Immunofluorescence staining in SAM patients with different severity showed a total or partial absence of DSG1. The exact pathological mechanisms and genotype-phenotype correlation for *DSG1* variants remain to be elucidated.

13 – Fieke Rosenberg

A genome-wide association study of hand eczema identifies locus 20q13.33 and reveals genetic overlap with atopic dermatitis

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Background Twin studies revealed that genetic effects play a role in hand eczema (HE), but the responsible genetic factors are unknown.

Objective Our primary aim was to identify and characterize genetic loci associated with HE. Furthermore, we aimed to provide more insight into the genetic overlap between HE and atopic dermatitis (AD).

Methods We performed a genome-wide association study (GWAS) of HE (Ncases=2,879) in Lifelines, a large population-based cohort and biobank study. In addition, a GWAS of AD (Ncases=1,706) and a genetic correlation analysis between HE and AD (independent datasets) were performed.

Results We identified multiple genome-wide significant variants at locus 20q13.33 associated with HE, regardless of adjusting for AD. The lead SNP (rs8114049) is an intron variant mapping to *RTEL1* and *RTEL1-TNFRSF6B* genes (p = 2.053 x 10^{-11}). When we adjusted for AD in the HE GWAS models, several variants remained significant and were previously associated with AD in other AD GWASs. This means locus 20q13.33 is pleiotropic for HE and AD. The region at locus 20q13.33 contains several immune regulation genes (*TNFRSF6B, ZGPAT, LIME1)* that may play a role in the pathogenesis of both HE and AD. We revealed a strong significant genetic correlation between HE and AD (r_g=0.71-0.81), even when HE was adjusted for AD (r_g=0.69). The GWAS of AD revealed four suggestive variants (p<5x10^{-07}) at three known loci: 1q21.3 (*FLG-AS1*, *FLG)*, 5q22.1 (*SLC25A46*, *TSLP),* and 11q13.1 (*OVOL1*).

Conclusion Our results provide insight into the genetic factors of HE and reveal a large genetic overlap with AD.

14 – Jannik Rousel

Characterization of facial seborrheic dermatitis using non-invasive multimodal methodology

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Background: Facial Seborrheic dermatitis (SD) is a skin disorder characterized by the presence of erythematous, flaky and itchy skin. Its pathogenesis appears multifactorial with I) a disbalanced immune system, II) Malassezia driven microbial involvement and III) skin barrier perturbations.
Objective: To investigate whether inflammation, microbial involvement or skin barrier dysfunction is more pronounced in SD.
Methods: The lesional and non-lesional skin of 37 patients with mild untreated facial SD was comprehensively and non-invasively assessed with optical coherence tomography (OCT) and standardized photography to image inflammation. Amplicon sequencing and Malassezia species identification was used for microbial profiling. The skin barrier was assessed by trans-epidermal water loss and ceramide profiling.
Results: Significant increases in lesional skin for epidermal thickness, epidermal blood flow and superficial roughness were observed by OCT and 2D-photography indicated significantly more erythema. While the abundance of *Staphylococcus* was significantly increased in lesional skin, abundance of *Malassezia* and *Malassezia* species were not significantly different. Lesional skin showed a significantly impaired skin barrier with significant underlying skewed ceramide subclass composition, impaired chain elongation and increased chain unsaturation. Changes in the ceramide profile correlated with the degree of barrier impairment. Integrative analysis shows two distinct populations after stratifying for lesional and non-lesional skin with chain length and increased ceramide [NS] as most discriminating features.
Conclusions: This study shows skin barrier dysfunction to be heavily implicated in SD. Barrier restoration might be a suitable target for future therapeutic options in the treatment of facial SD

15 – Coco Dekkers

Immunological changes in atopic dermatitis patients treated with different dosing intervals of dupilumab

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Background Dupilumab, a human IgG4 monoclonal antibody targeting the interleukin-4 receptor alpha (IL-4Rα), substantially improves disease severity in patients with atopic dermatitis (AD). A recent daily practice study has shown that dose reduction of dupilumab can be successfully applied in patients with controlled AD, but the associated biological effects are currently unknown.

Objective To study the T- and B-cell dynamics in moderate-to-severe AD patients after dupilumab interval prolongation.

Methods Peripheral blood mononuclear cells and serum were isolated from eleven AD patients who were treated with 300mg dupilumab subcutaneously every 2 weeks (Q2W), every 4 weeks (Q4W) and every 6 weeks (Q6W). Dupilumab serum levels were measured using liquid chromatography-tandem mass spectrometry. The IL-4Rα occupancy and (skin-homing) T-cell function were analyzed using flow cytometry. Total IgE levels were measured with ELISA.

Results During treatment with dupilumab Q2W and Q4W, the IL-4Rα expression was undetectable regardless of the number of days between the injection of dupilumab and blood draw. Simultaneously, the Eczema Area and Severity Index (EASI) score remained stably low and IgE levels declined. However, during treatment with dupilumab Q6W, the IL-4Rα became detectable again and in a subset of patients the percentage of proliferating (Ki67+) and Th2 cytokine producing skin-homing CD4+ T-cells increased. This was also reflected in the clinical parameters with a slightly rising EASI score.

Conclusion Dupilumab Q2W and Q4W stably inhibited IL-4Rα with similar immunological and clinical effects. The transition from dupilumab Q4W to Q6W seems to be an important biological tipping point for disease control.

16 – Marie-Eline Debeuf

Efficacy of Erbium:YAG laser therapy in morbus Hailey-Hailey: a prospective observational study

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Background Hailey-Hailey disease (HHD) is a rare genetic skin fragility disorder with great disease burden caused by variants in the *ATP2C1* gene. Er:YAG ablative laser therapy is a therapeutic modality with promising results.

Objective To analyse the effect of Er:YAG laser therapy on erosive plaques in patients with HHD and on patient's quality of life (QoL).

Methods In this prospective observational study, eight patients with HHD were included and treated with Er:YAG laser therapy. Skin biopsies were taken before laser therapy, six weeks after laser therapy and in clinically uninvolved skin to determine the number of desmosomes, intercellular distance and perinuclear retraction of intermediate filaments by electron microscopy. Comparison was made to skin samples of three individuals without HHD. Skindex-29 and DLQI questionnaires were used to evaluate QoL before laser therapy, six weeks after laser therapy as well as three years after laser therapy.

Results Hailey-Hailey plaques resolved completely following laser therapy, even at 3-year follow-up, and was associated with significant improvement in QoL. Electron microscopy results will be shared at the conference.

Conclusion Ablative Er:YAG laser therapy leads to long-term remission of M.Hailey-Hailey.

17 – Jill Olydam

Effectiveness of abrocitinib treatment in patients with difficult-to-treat atopic dermatitis: daily practice data

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Background Abrocitinib is a JAK-1 selective inhibitor registered for the treatment of moderate-to-severe atopic dermatitis (AD). Although efficacy and safety have been shown in phase 3 clinical trials, data on real-life patients with a treatment history of targeted-therapies are scarce.

Objective To evaluate the effectiveness and safety of abrocitinib treatment in patients with difficult-to-treat AD in daily practice.

Methods In this prospective observational single-center study, all AD patients who started abrocitinib treatment in the context of standard care between April 2021 until December 2022 were included. Effectiveness was assessed using clinician- and patient-reported outcome measures. Adverse events were evaluated.

Results Forty-one patients were included. Abrocitinib treatment resulted in a significant decrease of disease severity during a median follow-up period of 25 weeks (IQR 16-34). Median EASI score at baseline decreased from 14.7 (IQR 10.4-25.4) to 4.0 (IQR 1.6-11.4) at last review (p<0.001). Median NRS itch decreased from 7.0 (IQR 5-8) to 3.0 (IQR 1-2) at last review (p<0.001). A total of 30 patients (73.2%) had failed on previous targeted-therapies due to ineffectiveness, including JAK-inhibitors (n=14, 34%) and biologics (n=16, 39%). The most frequently reported AEs included gastrointestinal symptoms (27.6%), acne (20.7%) and respiratory-tract infections (17.2%). Sixteen (39%) patients discontinued abrocitinib treatment due to ineffectiveness, AEs or both (41.2%, 41.2% and 11.8%, respectively).

Conclusion Abrocitinib can be an effective treatment for patients with moderate to severe AD in daily practice, including non-responders on other targeted therapies.

18 – Sarah Thomas

Drug survival of IL-17 and IL-23 inhibitors for psoriasis: a systematic review and meta-analysis

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Background The most recently approved biologics for psoriasis are the interleukin (IL)-17 and IL-23 inhibitors. Drug survival is a frequently used outcome to assess drug performance in practice. An overview of the available drug survival studies regarding IL-17 and IL-23 inhibitors is lacking.

Objective To perform a systematic review and meta-analysis of drug survival of IL-17 and IL-23 inhibitors for psoriasis.

Methods A systematic review and meta-analysis was conducted by searching 4 databases until July 2022. Survival probabilities at monthly intervals were extracted from Kaplan-Meier curves using a semi-automated tool. Summary survival curves were constructed per biologic for different discontinuation reasons and split for the effect modifier biologic naivety. Results were analyzed separately for real-world patients data (registries/medical records) and for prescription data (claims/pharmacy).

Results Of 1310 abstracts screened for eligibility, 45 studies were included for analysis. Drug survival outcomes of 24,627 patients on secukinumab, ixekizumab, brodalumab, guselkumab and risankizumab were aggregated. Summary survival estimates of real-world studies for overall, ineffectiveness and adverse event related drug survival were high for all included biologics (all >0.80 at year 1). Guselkumab had highest five-year rates. Estimates of prescription databases were substantially lower than estimates from the primary analyses based on real-world data.

Conclusion This meta-analysis showed that investigated IL-17 and IL-23 inhibitors had high drug survival rates, with very high rates for long-term guselkumab drug survival. We showed that effect modifiers such as biologic naivety, and the source of data used (real-world vs. prescription databases) is relevant when interpreting drug survival studies.

19 – Reineke Soegiharto

Omalizumab drug survival for chronic urticaria – the DruSO-CU UCARE study

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Background Despite high efficacy of omalizumab in Chronic Urticaria (CU), long-term real-world data and drug survival studies in a large international population are lacking.

Objective To evaluate omalizumab drug survival and identify determinants in a UCARE cohort.

Methods Fourteen centres from ten countries included all CU patients treated with omalizumab. Drug survival, differentiated for discontinuation reasons, was analysed by Kaplan-Meier survival and determinants using Cox regression analysis.

Results 2325 CU patients (71% female; mean age 42 years) with a maximum omalizumab treatment duration of 12 years (4260 active treatment years) were included. Overall, drug survival rates were 76%, 60% and 43% after 1, 2 and 5 years, respectively (median drug survival: 3.3 years) and mostly determined by well-controlled disease. Omalizumab was discontinued by 890 (38%) patients, mostly (n=576, 65%) due to well-controlled disease, 18% (n=164) due to ineffectiveness and 4% (n=31) due to side-effects. Omalizumab was restarted in 253 (29%) patients, of which 90 (36%) discontinued. Short disease duration, absence of chronic inducible urticaria and fast response were independent determinants of shorter drug survival due to well-controlled disease. Immunosuppressive co-treatment at start of omalizumab, presence of autoimmune disease and absence of wheals were determinants of shorter drug survival due to ineffectiveness. Long disease duration and no fast response were determinants of shorter drug survival due to side effects.

Conclusion This study confirms the high effectiveness and safety in a large international multi-centre cohort. Knowledge of the determinants of drug survival supports predicting treatment duration and managing patients' and physicians' expectations.

20 – Celeste Boesjes

Influence of pathogenic filaggrin variants on dupilumab treatment in atopic dermatitis

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Background Pathogenic variants in filaggrin (FLG) are associated with an increased risk of atopic dermatitis (AD).

Objective To evaluate the influence of FLG variants on the effectiveness of dupilumab treatment in AD.

Methods This prospective observational study included adult AD patients treated with dupilumab from the BioDay Registry. FLG was analysed with smMIP targeted sequencing. Novel mutations were confirmed by Sanger sequencing. Eczema Area and Severity Index (EASI), Investigator Global Assessment (IGA), Numeric Rating Scale (NRS) pruritus, Dermatology Quality of Life Index (DLQI) and Patient Oriented Eczema Measure (POEM) were assessed at baseline and week 16/52.

Results Genetic analysis of 285 included patients showed bi-allelic mutations FLG-/- in n=41 (14%), heterozygous FLG-/+ in n=64 (23%) and FLG+/+ in n=180 (63%). Three novel mutations (p.E651*, c.8318del, c.10086del) were found. We observed fairly similar outcomes regarding EASI response, EASI≤7, mean EASI, NRS pruritus, IGA and total POEM scores for patients with and without pathogenic FLG variants at all time points. DLQI was significantly higher in patients with versus without FLG variants at week 52, and for FLG-/- versus the FLG+/+ and FLG-/+ groups at week 16. POEM flaking and dry skin showed significant higher scores at week 16 and 52 in patients with FLG variants compared to FLG+/+, with exception of POEM dry at week 52 with significance only between FLG-/- and FLG+/+ groups.

Conclusion AD patients with pathogenic FLG variants showed a similar physician-reported response to dupilumab treatment, while patient satisfaction was negatively impacted based on DLQI and POEM flaking/dry skin.

21 – Walbert Bakker

Analyzing resistance of AXL- and/or MITF-expressing melanoma cells to immunotherapy

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Background Tumor heterogeneity presents a hurdle to effective therapy as illustrated by "mixed responses" frequently observed in immunotherapy-treated patients. Previously, AXL+ melanoma cells were identified to be resistant to targeted therapy, whereas more differentiated MITF+ melanoma cells responded well.

Objective In this study we analyzed whether we could validate these AXL+ or MITF+ melanoma subpopulations at the protein level, and explored if they respond differentially to immunotherapy,

Methods Subpopulations were identified using multiplex immunofluorescence. Immunotherapy was applied either by immune checkpoint inhibition (anti-CTLA-4) or autologous tumor lysate-loaded dendritic cell vaccination. The R2 Genomics Analysis and Visualization platform was used to analyze AXL and MITF expression in the cutaneous melanoma TCGA dataset, and in a recently published single-cell RNAseq dataset.

Results Our data demonstrate large inter patient variability, and variable changes in tumor subpopulations, independent of the type of immunotherapy. Immunotherapy did not select for AXL- cells. Immunotherapy-induced changes in the abundance of AXL+ MITF- cells did not correlate with survival. However, MITF+ tumor cells showed a statistically significant inverse correlation with CD8+ T cell infiltration. Furthermore, we confirmed the reported negative correlation between *AXL* and *MITF* mRNA expression in bulk tumors, but not at the level of melanoma cells, indicating that this association extends beyond melanoma cells. Indeed we confirmed the expression of AXL in cancer associated fibroblasts, and showed significant AXL expression in macrophages.

Conclusion Our study highlights that AXL+ tumor cells are not solely melanoma cells, and may not be as resistant to immunotherapy as to targeted treatment.

Knockout of JAK/STAT signaling inhibitors in skin-resident CD4+ T cells results in an autonomous skin inflammation with features of early stage mycosis fungoides

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Background Recent detailed genomic analysis of CTCL identified genes acting as JAK/STAT inhibitors as frequently deleted tumor suppressors and a one-copy deletion of one of these genes was confirmed in early-stage mycosis fungoides (MF) lesions.

Objective and Methods For a better understanding of the functional role of these genes in the genesis of MF, we developed a genetically engineered mouse model simulating loss in skin resident CD4 T cells. This was followed by experimentally induced sustained skin inflammation for 20 weeks and 10 weeks without further treatment.

Results The mice with one-copy deletion of one of these genes in skin resident CD4 T cell showed more CD3, CD4 and CD8 cells in the dermis, more Stat3 activation of T cells in the skin and one mouse developed signs of a noticeably thicker epidermis, massive inflammatory cell infiltration and increased Stat3 activation in T cells in both epidermis and dermis.

Conclusion We show that mice with one-copy deletion of a specified gene encoding a JAK/STAT inhibitor in skin resident CD4 T cells in combination with a protracted contact allergic reaction displayed autonomous inflammatory responses and features of early-stage MF.

23 – Shidi Wu

Distinct roles of human papillary and reticular fibroblasts on tumor cell invasion and the underlying therapeutic values in tumor microenvironment-targeting therapy

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Background Normal human dermal fibroblast (NF) can be morphologically divided into papillary fibroblast (PF) and reticular fibroblast (RF). Dysregulated NF activation is one major origins of cancer associated fibroblast (CAF) in solid tumors. However, the heterogeneity of NF during this activation process is poorly studied.
Objective To dissect the discrepancy between PF, RF and CAF and their distinct roles in tumor cell invasion, followed by developing therapeutic strategies guiding a CAF normalization process.
Methods We compared the morphology, growth curve and transcriptome in the three fibroblast subtypes in 2D cultures, followed by establishing 3D organotypic cultures mimicking melanoma, cSCC, vSCC and HNSCC by seeding corresponding tumor cell lines on dermal matrixes populated with PF, RF or CAF. Additionally, chemotherapeutics, pathway inhibitors and industry-derived compounds were applied to observe their effects on CAF phenotypes and tumor-stroma crosstalk.
Results In monolayer, we identified markers predominantly expressed in each fibroblast subtype respectively. 3D co-culturing tumor cells with RF led to enhanced tumor invasion via EMT and profound CAF marker expression (α-SMA, COL11A1) in the dermal compartment compared to PFs, indicating RF to be the precursor of CAF. Several inhibitors and compounds were screened and significantly decreased the expression of up-mentioned CAF markers and cytokines (IL-6, CXCL12) as well as inducing a PF phenotype in CAF in both 2D and 3D cultures.
Conclusion We report distinct roles of PF and RF on tumor invasion and propose a "CAF to PF" normalization process which provide new insights in tumor microenvironment-targeting therapy.

24 – Yang Liu

Screening and validating key genes in MF that promote T cell malignant transformation through single-cell datasets and whole-genome sequencing

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Background Mycosis fungoides (MF) constitutes the most frequent cutaneous T cell lymphoma. It is worth paying attention to the changes in gene expression that occur as T cells transform from a benign to a malignant phenotype during the early and advanced stage of MF.

Objective To identify the key genes that drive the malignant transformation of T cells and investigate how these genes regulate the transformation. Furthermore, we aim to explore whether these key genes influence the tumor microenvironment through cancer-associated fibroblasts and macrophages, thereby promoting the malignant transformation of T cells.

Methods We used the GEO database to obtain single-cell sequencing data from early and advanced stages of MF and searched the Genecards database for genes related to the malignant transformation of T cells. tSNE was used to identify the spatial relationship between these genes and various cell types, including T cells, cancer-associated fibroblasts, and macrophages. Subsequently, our own whole-genome sequencing data was applied for the validation of key genes.

Results The single-cell data showed that genes related to the malignant transformation of T cells were mainly distributed in the tSNE map of T cells, macrophages, and fibroblasts. Pathway enrichment analysis showed that these genes were mainly involved in lymphocyte malignant proliferation and abnormal T-cell receptor signaling.

Conclusion Key genes may promote the transformation of T cells from a benign to a malignant phenotype through the tumor microenvironment, including macrophages and fibroblasts. This provides new insights into the malignant progression and treatment of MF.

POSTERS

P1 – Miranda Jekhmane

Towards Native Skin: ex vivo development of skin appendages

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Background Skin defects resulting from burns, trauma or chronic wounds are a major burden. Split-thickness skin autografting represents the clinical gold-standard, but has limitations that call for alternatives. Other treatments have failed to recapitulate skin in form and function due to the absence of skin appendages. Dermal papilla (DP) cells in hair follicles (HF) bear hair-inductive characteristics and provide an accessible option to engineer a full skin equivalent (FSE) that extends beyond wound healing. DP cells generate hair growth when implemented in vivo in animal models. The successful translation of such strategy to humans may pave a way for a broad clinical application of skin tissue engineering.
Objective Our study aims on using DP cells to generate FSE's with skin appendages.
Methods DP cells were isolated from human HF using an enzymatic digestion procedure. To preserve the hair-inductive characteristics, spheroids of DP cells were produced using the hanging drop method. Flow cytometry, RNA analysis and immunohistochemistry were used to characterise the cells in 2D and 3D format.
Results DP cells were successfully isolated and cultured in 2D format. Characterization of the cells revealed expression of several cellular markers supporting the stem cell activities of DP cells in wound healing. Our 3D culture indicated that cell passage is a critical factor for spheroid formation with smaller sized and less uniform spheroids observed above cell passage 5.
Conclusion We defined a reproducible isolation method that harbours a viable and proliferative 2D and 3D culture system of DP cells.

P2 – Britt van der Leeden

Systemically increased neutrophil activity and altered coagulatory phenotype after burn injury

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Background Burn injury induces a prolonged local and systemic inflammatory response that often is accompanied by local intravascular coagulation. Previously we have shown that neutrophil extracellular traps (NETs) are locally present in microcirculatory thrombi in burn wounds which coincided with a switch in the microcirculatory endothelium toward a procoagulant phenotype.

Objective This study aimed to investigate the systemic neutrophil response and procoagulatory effects in time after burn injury.

Methods Plasma was obtained from burn patients (n=7; mean total body surface area of 35% burned) at different time points post-burn (1-15 days) and healthy controls. Herein, the levels of neutrophil activation marker (HNE-a1ATC), DNA cleaving protein (DNAse1), nucleosome complex for extracellular DNA and MPO bound to extracellular DNA for NETs, as well as the coagulatory markers prothrombin factor 1.2 (PTF12), thrombin anti-thrombin complex, tissue plasminogen activator (tPA), and plasminogen activator inhibitor 1 (PAI-1) were determined by ELISA. Additionally, correlation analyses between these plasma levels were performed.

Results Increased plasma levels of HNE-a1ATC, DNAse1, nucleosome complex NETs, tPA, and PAI-1 were found in burn patients compared to controls. HNE-a1ATC, NETs, and PTF12 were significantly correlated to timing after burn injury. Levels of nucleosomes and PTF12 significantly correlated with burn wound size. Burn wound size and depth, plasma nucleosome and PAI-1 levels significantly correlated to plasma C-reactive protein levels.

Conclusion Our study shows a systemic increase in neutrophil activity, NET formation and coagulation after burn injury with no clear effect over time, that may be caused by the previously observed local intravascular coagulation.

P3 – Wouter Ouwerkerk

Biomarkers for early detection of re-pigmentation in patients with non-segmental vitiligo after standard-care treatment

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Background The treatment of non-segmental vitiligo (NSV) remains challenging. Aside from a delay in visible repigmentation during therapy, it is uncertain whether a patient will ultimately respond to treatment. An early on treatment biomarker could help to predict the response to therapy.

Objective This study aimed to evaluate the association between early changes within various immuno-cell types (T cell subsets and NK cells in blood and skin) and clinical response to treatment.

Methods This study prospectively included NSV patients, starting with standard of care treatment consisting of topical therapy alone or in combination with narrowband UVB phototherapy. Evaluation of repigmentation was done by comparing Vitiligo Extent Score at baseline and 6 months after starting therapy. Blood samples and (non)-lesional skin biopsies were taken at baseline and 3 months after therapy. These samples were analysed for changes in cells expressing CD3, CD4, Granzyme B+ (GrB)+CD4, CD8, GrB+CD8, GrB+CD8-, TRM, Treg, NK and GrB+NK cells at baseline and 3 months and associated to repigmentation.

Results A total of 30 patients completed the study. Our results showed that early increase of GrB+CD8- T cells in skin was associated to repigmentation. In blood, changes in GrB+CD4 T cells were associated with repigmentation. Similar relations with GrB+NK cells were found in skin.

Conclusion This study evaluated changes in cellular immunity shortly after standard of care therapy in relation to repigmentation. Both adaptive and innate immuno-changes play a role in response to treatment. Future studies need to evaluate clinical useability of these biomarkers.

P4 – Elisabetta Michielon

An organotypic human melanoma-in-skin model as in vitro tool for testing cell-based therapies

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Background Despite recent extraordinary clinical success in treating melanoma with immune checkpoint blockade, a majority of patients do not respond or develop adaptive resistance resulting in poor prognosis. Therefore, a clear need exists for additional therapy options. The ability of Vγ9Vδ2-T-cells to recognize and kill transformed cells independently of HLA-matching makes them a promising candidate for immunotherapy. In vitro melanoma reconstructed human skin (Mel-RhS) models have been developed to mimic features of melanoma progression and thus have an attractive potential to test melanoma-targeted therapies in preclinical studies.

Objective Investigating the capacity of Vγ9Vδ2-T-cells to target tumors in Mel-RhS.

Methods A375 melanoma cells and keratinocytes were co-seeded onto fibroblast-populated dermal equivalents (Mel-RhS). Expanded peripheral blood-derived Vγ9Vδ2-T-cells were injected into Mel-RhS or healthy controls (RhS), assessed for activation markers by flow cytometry, and their tissue location was identified by immunohistochemistry.

Results Vγ9Vδ2-T-cells were viable up to three days in (Mel-)RhS and cells in contact with the tumor acquired the Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP) antigen from the melanoma cells. MCSP+ (i.e. cross-dressed) Vγ9Vδ2-T-cells expressed higher levels of the activation markers 4-1BB and NKp44 compared to those from RhS or their MCSP- counterparts. CXCL10 secretion, involved in T-cell recruitment into the tumor, was upregulated in supernatants from Vγ9Vδ2-T-cell-containing Mel-RhS, compared to without Vγ9Vδ2-T-cells.

Conclusion A fraction of Vγ9Vδ2-T-cells injected into Mel-RhS came into close contact with the melanoma cells, acquired melanoma-associated membrane antigens, likely through trogocytosis, and became selectively activated. The novel Mel-RhS may present a promising tool to test T-cell-based therapies.

P5 – Selinde Wind

Shift in early-stage mycosis fungoides tumor micro-environment induced by topical chlormethine by flow cytometry of interstitial fluid

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Background Early stages of Mycosis fungoides (MF) can be treated effectively with topical chlormethine. However, insight into changes in the tumor-micro environment (TME) during treatment and how these changes contribute to therapeutic success is limited.

Objective In this study we aimed to characterize TME of MF on a cellular level by suction blister fluid analysis.

Methods In this exploratory, open-label, deep phenotyping trial a total of 21 early-stage (Ia/Ib) MF patients were treated with chlormethine gel 160µg/g QD for 16 weeks. Suction blister exudates were collected pre-treatment from lesional (LS) and non-lesional skin (NL) and after 16 weeks of treatment from LS and analyzed with flow cytometry. Here, we present preliminary data from the first 18 completers of the trial. For all statistical analyses a Wilcoxon signed-rank test was used.

Results In blister exudate pre-treatment, significantly more absolute cells were observed in LS compared to NL ($p<0.001$). Exudates from LS contained significantly more CD3+ cells ($p<0.001$), CD3+4+ T-lymphocytes ($p<0.0001$), activated CD4+HLA-DR+ effector T-lymphocytes ($p=0.0001$), CD3+8+ T-lymphocytes ($p<0.0001$), activated CD8+HLA-DR+ cytotoxic T-lymphocytes ($p<0.001$), CD14-CD16- dendritic cells ($p<0.01$) and CD68+ macrophages ($p<0.01$). After 16W chlormethine gel treatment significantly less aberrant T-cells ($p<0.05$), CD3+8+ T-lymphocytes ($p<0.05$), activated CD8+HLA-DR+ cytotoxic T-lymphocytes ($p<0.01$) and Tregs ($p<0.05$) were observed compared to LS baseline.

Conclusion We show for the first time the feasibility of suction blister fluid analysis to investigate TME in MF patients. These preliminary results suggest that CD8+HLA-DR+ cytotoxic and regulatory T-lymphocytes have a prominent role in chlormethine therapy in MF.

P6 – Laura van der Meulen

Topical clobetasol-induced changes in the cutaneous microbiome composition of lichen sclerosus

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Background Dermatological clinical trials frequently analyse the microbiome composition as either clinical endpoint or as exploratory biomarker. New compounds are being developed targeting dysbiosis for a range of dermatological and gynaecological conditions. However, little is known about the microbiome-modulating effects of topical corticosteroids, dermatology's golden standard.
Objective The aim of this study was to investigate the effects of topical clobetasol on the microbiome composition of patients with vulvar lichen sclerosus.
Methods The effect of clobetasol on the microbiome composition was analysed in patients with lichen sclerosus in a prospective, open-label clinical trial. Women with vulvar lichen sclerosus (n=10) were enrolled. Swabs of the vulva were collected and the microbiome composition was characterized with metagenomic shotgun sequencing. Clobetasol was applied on the affected vulvar area for 4 weeks. Pre- and post-dose samples were analysed.
Results Lactobacillus, Prevotella and Finegoldia were the most abundant taxa of vulvar LS skin at baseline. Treatment with clobetasol did not yield significant changes in the microbiome composition of vulvar skin, although the proportion of Lactobacillus and Staphylococcus increased, while the relative abundance of Prevotella and Finegoldia appears reduced after treatment.
Conclusion No alterations to the vulvar microbiome composition were observed after 4-week clobetasol treatment of lichen sclerosus. We conclude that corticosteroids do not directly affect the microbiome composition of the vulvar skin. However, indirect, long-term immunomodulatory effects cannot be ruled out at this stage. These findings raise questions for the applicability for new compounds targeting dysbiosis in lichen sclerosus.

P7 – Tessa Goedhart

The development of personalised treatment for patients with chronic skin diseases through deep phenotyping: an overarching study protocol for multi-center observational trials

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Background Chronic skin diseases have a major socio-economic impact since prevalence is high and patients' quality of life can be low. Current dermatological therapeutic strategies are often insufficient due to the 'one size fits all' nature of patient care. By constructing biomarker profiles for mycosis fungoides (MF), hidradenitis suppurative (HS), chronic spontaneous urticaria (CSU), cutaneous lupus erythematosus (CLE), atopic dermatitis (AD), and psoriasis vulgaris (PV), targeted personalized treatment strategies can be developed.

Objective This poster describes an overarching deep phenotyping study design to analyse MF, HS, CSU, CLE, AD and PV in the Netherlands.

Methods Per chronic disease, a multi-centre, non-randomised, controlled, open-label, observational study will be conducted over the course of 52 weeks. Thirty-two patients diagnosed with one of the six chronic skin diseases and eight healthy volunteers, aged 18 years or older, will be included in each study.

Clinical parameters will be evaluated two weeks before start of treatment, at baseline and at week 4, 8, 12, 16, 24, and 52 via clinical assessments, imaging (i.e. Laser Speckle Contrast Imaging, Antera 3D camera, full-body photography), patient-reported outcomes, biopsy and microbiome swabs. Blood samples will be taken at baseline and at week 4, 12, and 24 after treatment initiation for biomarker analyses.

Results This study design will provide a comprehensive dataset per chronic skin disease with i.e. molecular, and genetic biomarkers.

Conclusion Overall, this study aims to provide a comprehensive understanding of chronic skin disease via deep phenotyping, with an emphasis to standardize targeted personalized treatment.

P8 – Anne Schlösser

Tralokinumab for patients with moderate-to-severe atopic dermatitis: results from daily practice

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Background Evidence about tralokinumab treatment for moderate-to-severe atopic dermatitis (AD) in daily practice is limited.

Objective To report the first evidence, from daily practice treatment with tralokinumab in patients with AD.

Methods Thirty seven AD patients who received tralokinumab treatment in the context of routine care at the Erasmus Medical Centre were included. 28 patients had previously been treated with dupilumab, and 14 patients who had been treated with a Janus kinase inhibitor (JAKi). The Investigator's Global Assessment (IGA; 0–4) and the numeric rating scale peak pruritus during the past 7 days (NRS itch 7d: 0–10), adverse events and reasons for discontinuation were analysed. A good clinical response was defined as any decrease in IGA and NRS itch 7d, and patient was satisfied with the treatment and wished to continue with therapy.

Results In total, 37 patients were treated with tralokinumab. Twenty-two (59%) patients showed a good response to tralokinumab treatment. Fifteen (41%) patients discontinued treatment because of inadequate AD control or adverse events. Treatment-related adverse events were mild in most patients. Half of the patients that failed on dupilumab showed a good clinical response to tralokinumab.

Conclusion Tralokinumab was found to be effective in most patients in this cohort with difficult-to-treat, severe AD from daily practice. Interestingly, tralokinumab was also found to be effective in 50% of patients who had previously experienced insufficient response or adverse events with dupilumab treatment.

P9 – Willemijn Witkam

The epidemiology of acne vulgaris in a multi-ethnic adolescent cohort: a cross sectional study

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Background Acne vulgaris (AV) is a chronic multifactorial inflammatory disease, often in adolescents, with a high psychological burden.

Objective To study the prevalence and determinants for AV in a large multi-ethnic cohort at the start of puberty.

Methods This cross-sectional study is embedded in Generation R, a birth cohort study from Rotterdam, the Netherlands. 3D facial photos taken at the center visit in 2016-2019 were used to grade AV severity using the Global Acne Severity scale by two physicians. Demographic, lifestyle factors and host's factors were compared between acne severity (clear/almost) clear skin; mild and moderate/severe) using multivariable ordinal logistic regression and stratified across sex.

Results 4561 subjects (51% girls) were included in the analysis with median age of 13.5 (IQR 13.3-13.6). The AV prevalence (total of mild + moderate/severe cases) girls vs. boys was 62% vs. 44.7% and specifically for moderate/severe AV was 14% vs. 8.7%. The proportion of girls and boys who had AV and received treatment were low (19.0% and 14.0%). Higher puberty stages (ORs: 1.38 (1.20 - 1.59)) and 2.16 (1.86 - 2.51) for girls and boys) and darker skin colors V and VI (ORs: 1.90 (1.17 - 3.08) and 2.43 (1.67 - 3.56 for girls and boys respectively) were predictors for both sexes, being overweight only for boys (1.58 (1.15 - 2.17)).

Conclusion AV prevalence was already high, often not treated at age of 13 and related to sex, puberty status, darker skin color and weight status.

P10 - Maria Jeanine Medendorp

Atypical Fibroxanthoma and Pleomorphic Dermal Sarcoma: a clinicopathological study

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Background Atypical fibroxanthoma (AFX) and pleomorphic dermal sarcoma (PDS) are rare cutaneous neoplasms showing many similar clinicopathological features. AFX usually behaves benign and PDS can behave malignant and can metastasize. To date, little is known about these entities and there is no consensus about safe excision margins.

Objective To describe clinicopathological characteristics distinguishing AFX and PDS and evaluate safety of excision margins; respectively 5mm and 1 cm.

Methods Data from electronic patients files was collected from all AFX or PDS diagnosed between 2012-2018 and histology was revised by a pathologist specialized in soft tissue pathology.

Results Of 49 tumors: 16 (33%) AFX, 25 (51%) PDS and 8 (16%) AFX/PDS (no conclusive diagnosis). Comparing AFX and PDS, patients with PDS were 6 years older of age (p=0.02). Furthermore, the difference in diameter at clinical examination and histologic examination was greater in AFX than PDS (p=0.03), suggesting an underestimation of clinical diameter of the AFX by the clinician compared to PDS. We report 4 recurrences after complete excision, solely of AFX. None of the patients in our study population had metastases.

Conclusion PDS patients are older than AFX, but otherwise show no differences in clinicopathologic features. The remarkable underestimation of tumor size in AFX tumors together with 4 recurrences of AFX, suggests that an excision margin of 5 mm is too small. The 1 cm excision margin for PDS seems safe.

P11 – Roderick Slieker

P16 loss increases genetic dependency for mitochondrial genes in melanoma

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Background The p16INK4a-CDK4/6-Rb pathway is dysregulated in many cancers. Both the p16 and p14 tumor suppressor proteins are encoded by the CDKN2A gene and are frequently mutated across tumor types. Pathogenic gene variants in CDKN2A are the cause of hereditary melanoma and pancreatic cancer. Patients harboring CDKN2A mutations are therefore monitored throughout life for the onset of melanoma. As such, identifying vulnerabilities specific for cancers with p16 loss in particular melanoma is key.

Objective To identify genetic and drug vulnerabilities in p16 deficient (p16-) cancers and in particular melanoma.

Methods Genetic dependency and drug screen data of 1436 cell lines across 31 lineages were obtained from DepMap (v22Q4). Cell lines were considered p16 deficient in the case of: loss of the CDKN2A locus, presence of a mutation or absent p16 mRNA expression. Differences in genetic dependency and drug response were investigated between p16+ and p16- groups.

Results P16 loss was associated with increased dependency on G1-phase genes, while less dependent on genes involved in the G1/S transition (Reactome, PFDR= $5.1 \cdot 10-16$). While p16 inhibits both CDK4 and CDK6, p16- cell lines were only dependent on CDK6 (β=-0.5, PFDR=$2.9 \cdot 10-36$) and not CDK4 (β=0.04, P¬FDR=0.64) compared to p16+. Exclusively in melanoma, increased genetic dependency was enriched for Mitochondrial Translation Elongation (Padj=$1.3 \cdot 10-27$) and Respiratory Electron Transport (Padj=$6.7 \cdot 10-22$) encompassing genes encoding mitochondrial ribosomal proteins and mitochondrial cytochrome c oxidase proteins.

Conclusion P16 loss was associated with increased dependency on CDK6 but not CDK4. In melanoma, p16 loss was associated with increased dependency on mitochondria-related genes.

P12 – Juliette Kersten

An overview of the epidemiology and therapeutical modalities in 426 patients with Mycosis Fungoides and Folliculotropic Mycosis Fungoides in the Netherlands

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Background The most common type of cutaneous T-cell lymphomas (CTCLs) is Mycosis Fungoides (MF). Folliculotropic Mycosis Fungoides (FMF), recognized as a distinct variant of MF in the World Health Organization-European Organization for Research and Treatment of Cancer (WHO-EORTC) classification, is less prevalent and has different clinical features.
Objective This study aimed to assess and compare patient attributes, disease advancement and treatment modalities for different stages of MF and FMF in Dutch patients.
Methods The registry of the Dutch Cutaneous Lymphoma Group was used to collect clinical and follow-up data from 426 patients diagnosed with MF or FMF between 2000 and 2019. The diagnosis was confirmed by a group of experts consisting of dermatologist and pathologists during regular meetings of the Dutch Cutaneous Lymphoma Working Group based on the clinicopathological criteria provided by the WHO-EORTC classification. Patients were classified as and included with early and advanced stage disease, and variables such as age, sex and therapy were recorded.
Results This study included 316 patients diagnosed with MF and 110 patients diagnosed with FMF. Our findings reveal a worse prognosis for advanced disease stage compared to early disease stage. Compared to MF, therapeutical modalities in FMF required more frequent changes. Notably, all patients included in this study were treated with topical corticosteroids at some point during their disease course.
Conclusion In comparison to FMF, MF has a higher prevalence. Patients with early stage disease have better outcomes than those with advanced stage disease. This study identified differences in the treatment approach between MF and FMF.

P13 – Fauve van Veen

Exploring perceived quality of life in middle-aged to old-aged patients with inherited ichthyosis

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Background Inherited ichthyosis refers to a rare group of keratinization disorders caused by genetic variants characterized by scaling and erythema. Ichthyosis patients suffer from physical and mental complaints affecting their quality of life (QoL). There is limited knowledge regarding the influence of ichthyosis on the QoL of adult and older patients.
Objective To investigate the impact of ichthyosis on the biological-psychological-social QoL of adults and older patients.
Methods We performed semi-structured interviews with participants diagnosed with inherited ichthyosis. Specific topics concerning the biological-psychological-social QoL, were questioned using an interview protocol. All interviews were recorded, transcribed, and analyzed using grounded theory. Validated QoL questionnaires (Dermatology Life Quality Index (DLQI) and Skindex-29) were collected.
Results Sixteen participants were interviewed with a diagnosis of autosomal recessive ichthyosis, bullous congenital ichthyosiform erythroderma, or X-linked ichthyosis. We learned that ichthyosis has an impact on the biological, psychological and social quality of life of these patients.
Conclusion We found a negative impact of ichthyosis on QoL and proposed several suggestions for healthcare improvements.

P14 – Emmy Crüts

Different surgical margins in T1 squamous cell carcinomas of the lip: a Dutch retrospective multicenter cohort study

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Background Carcinomas of the lip are considered both skin and oral tumors, and are therefore treated by different specialties. There is currently no consensus regarding appropriate surgical margins for excision of a T1 squamous cell carcinoma (SCC) of the lip and existing guidelines vary. In the Netherlands, patients with T1 lip SCC are treated by excision with either a 5mm or a 10mm clinical tumor-free margin.
Objective To compare risk factors for recurrence and/or metastasis between patients with T1 SCC on the lip referred for excision with 5mm or 10mm clinical margins.
Methods Patients with T1 lip SCCs surgically treated between 2010 and 2018 at one of the four Dutch participating centers were identified using the Dutch Pathological Anatomical National Automated Archive. Relevant data on patient and tumor characteristics were obtained retrospectively from patient records.
Results A total of 240 patients were included for analysis. Respectively 173 and 67 patients were treated with a 5mm and 10mm clinical margin. Results will be discussed during the NVED Annual Meeting 2023.
Conclusion The conclusion of this study will be discussed during the NVED Annual Meeting 2023.

P15 – Tristan Bruijn

Domatinostat-induced cutaneous toxicities in neoadjuvant treatment for stage III melanoma

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Background Stage III melanoma patients with a low tumoral interferon-gamma (IFN-γ) signature expression have demonstrated less favorable response rates upon neoadjuvant immune checkpoint inhibitor (ICI) therapy. Supplemental domatinostat (a class I histone deacetylase inhibitor) to nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) was hypothesized to lead to an increased anti-tumor immune response. However, unexpected domatinostat-specific dermatologic adverse events (DAE) hampered domatinostat dose escalation.
Objective To assess clinical and histologic patterns of domatinostat-induced cutaneous toxicity.
Methods The DONIMI trial was a two-center phase 1b trial testing IFN-γ signature-driven neoadjuvant combinations of domatinostat with nivolumab ± ipilimumab in adult (≥ 18 years) patients with stage III melanoma. Experienced dermatologists and pathologists reviewed laboratory testing, clinical pictures, and skin biopsies of DAEs.
Results Eight out of 40 patients developed DAEs consisting of prodromal systemic symptoms including fever, malaise, headache and abdominal complaints followed by a generalized maculopapular rash that typically covered >30% of the body surface area (grade 3 CTCAEv5.0).Two patients had raised liver enzymes. There were no signs of eosinophilia or atypical lymphocytes in peripheral blood. Symptoms had an onset of 10-12 days after treatment initiation and were managed by systemic corticosteroids and permanent cessation of domatinostat. Histopathological assessment revealed a vacuolar interface dermatitis with apoptotic keratinocytes and a superficial perivascular lymphocytic infiltrate without eosinophils, in some cases accompanied by a small vessel vasculitis.
Conclusion New ICI treatment combinations including domatinostat may lead to unexpected dermatologic toxicities with different clinical presentations than ICI-associated dermatitis or classic drug reaction with eosinophilia and systematic symptoms (DRESS).

P16 – Nika Kotnik

Infiltration analysis of activated, IL-31 and IgE expressing eosinophils and basophils in bullous and non-bullous cutaneous pemphigoid

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Background Itch and blister formation, the main symptoms of the autoimmune disease bullous pemphigoid (BP) are probably triggered by activated eosinophils and basophils migrating into the skin. Approximately 20% of BP patients do not develop blisters and this subtype of BP is called non-bullous cutaneous pemphigoid (NBP). Due to a variety of similar symptomatology, the differential diagnosis of NBP is often made late.

Objective In this retrospective study we analysed 31 skin sections from patients with BP lesional (BPles) and peri-lesional (BPperi) and NBP to address the question whether the different inflammatory pattern regarding basophils and eosinophils and state of activation plays a role in BP pathogenesis.

Methods We analysed the total number of eosinophils and basophils per area (mm2), the co-expression of IL-31, IgE, and the number of activated basophils (CD63+) and eosinophils (CD69+) in skin samples, which were scanned and automatically analysed by the software Fiji.

Results In our study we could show that both the number of eosinophils and basophils was markedly higher in BPles skin compared to BPperi and NBP skin. Eosinophils, as well as basophils in BPles skin were highly activated. In addition, IL-31+ and IgE+ eosinophils and basophils were seen in significantly higher numbers in BPles skin compared to BPperi and NBP skin.

Conclusion This finding underlines the role of both eosinophils and basophils in BP and gives first insights in the different inflammatory pattern of BP and NBP skin. Further research is needed to identify the complete mechanisms involved in skin blistering.

P17 – Rindert Venema

Delivery of therapeutic antisense oligonucleotides for exon skipping in epidermolysis bullosa

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Background Dystrophic epidermolysis bullosa (DEB) is a severe form of epidermolysis bullosa (EB) caused by mutations in the COL7A1 gene, encoding collagen type VII (C7). C7 is the major component of anchoring fibrils that form the structural connection between the basement membrane zone and the papillary dermis. In DEB, the absence of C7 leads to severe blistering of skin and mucosa. Currently, there is no cure for DEB, however, a promising therapy, antisense oligonucleotide (AON) mediated exon skipping, has shown to restore C7 expression in vivo and in vitro. Currently, a total 15 AONs have been approved and proven successful for disorders of similar etiology to DEB like Duchenne muscular atrophy.

Objective We investigate and optimize delivery of AONs in EB keratinocytes and fibroblasts to gain insights in its cellular uptake, distribution and effectiveness. Next, we will optimize transfection of single AONs in keratinocytes, and subsequently perform an 'oligowalk' in which we will transfect 121 different AON constructs specifically targeting exon 105 of the COL7A1 gene, to identify the most potent AON.

Methods Fluorescently labeled AONs were conjugated to our delivery compound and used to treat both healthy and patient keratinocytes followed by fluorescence microscopy-analysis and RNA-analysis. In parallel, the 'oligowalk' is performed by transfecting healthy primary keratinocytes and fibroblasts, using lipofectamine and polyethyleneimine.

Results/conclusion In preliminary experiments, we observed cellular binding, internalization and distribution of the AON construct by fluorescence microscopy. At the NVED we will present the data gathered in the coming months.

P18 – Duco Kramer

High resolution microscopic mapping of the basement membrane zone for better diagnosis of pemphigoid diseases

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Background Bullous pemphigoid is caused by autoantibodies against proteins in the basement membrane zone (BMZ). Sometimes, regular diagnostic techniques, e.g. direct- and indirect immunofluorescence, or biochemical analysis provide insufficient evidence for diagnosis. Fluorescent overlay antigen mapping can aid in the diagnosis. Fine mapping of the skin region with deconvolution imaging brings this diagnostic test to the next level. After locating various epitopes, we show the precise architecture of the BMZ. With this knowledge, we can locate the exact location of deposition of autosomal Ig's.

Objective We present a method to improve image analysis by implementing deconvolution in combination of statistical analysis and examination of the fluorescent signal.

Methods Cryosections of BP-patients or healthy individuals were triple stained for human Ig and collagen VII and Laminin 332 and/or BP180 and collagen IV. Imaging is performed on the Leica SP8X confocal microscope in deconvolution mode. Analysis of the BMZ is performed in ImageJ-software. The distance between the epitopes is calculated from the signal maxima.

Results We generated a map of several epitopes of proteins in the BMZ-region. The height of autosomal Ig signal corresponded with the type of pemphigoid disease, suggesting the method was valid. Here, we provide detailed insight into the structure of the BMZ.

Conclusion Deconvoluted high-resolution imaging of DIF biopsies aids the diagnosis of pemphigoid disease.

P19 – Joost Meijer

Gene expression profiling points to complement activation as important for blister formation in bullous pemphigoid

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Background One-in-five patients with the autoimmune bullous disease bullous pemphigoid (BP) present with severe pruritus without skin blisters (nonbullous pemphigoid, NBP), however, it remains unclear why the latter group lacks blisters.

Objective To assess the transcriptomics of inflamed lesional skin of BP and NBP patients and compare patient groups.

Methods Quantification of 180 gene transcripts associated with innate and adaptive immune responses of 12 BP, 12 NBP and seven control pruritus skin biopsies was performed by using the nanoString nCounter Myeloid Innate Immunity Panel (nanoString Technologies, Seattle, WA).

Results Data is presented in heatmaps, created using unsupervised clustering. Genes related to complement activation were highly expressed in 7/12 (58%) BP biopsies, in 1/7 (14%) control biopsies, but not expressed in any NBP biopsy (0/12). Furthermore, all BP biopsies showed a strong dual Th1 and Th2 response, while in NBP biopsies a dual Th1 and Th2 related gene expression was observed in 4/12 (30%) biopsies. Five (5/7) control pruritus samples showed dual high expression for Th1 and Th2 related genes, one NBP (1/12) and one control (1/7) sample showed high Th2 gene expression only.

Conclusion Taken together, exploring transcriptomics of inflamed lesional skin biopsies of BP and NBP patients revealed a difference in complement related gene expression between BP (highly expressed) and NBP biopsies (not expressed), supporting the hypothesis that complement activation plays an important role in blister formation.

P20 – Cisse Vermeer

An advisory on skin xenografts in single cell RNA sequencing

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Background Single-cell RNA sequencing (scRNA-seq) is a relatively novel technique that has established itself as a vital method to acquire detailed information on RNA expression profiles in individual cells or tissues of interest to better understand their biology and function.

Objective Our goal was to explore the applicability of scRNA-seq in investigating effects of antisense oligonucleotide-mediated exon skipping in human skin xenografts.

Methods Xenografts were generated from patient keratinocytes and fibroblasts harboring COL7A1 null variants on immunocompromised nude mice. After harvesting, xenografts were dissociated for use in scRNA-seq. Tissue dissociation is the process of obtaining live single cells in suspension from whole tissue. A dissociation protocol should be optimized for each type of tissue, and yield and viability of the resulting cells verified.

Results the optimized dissociation protocol resulted in acceptable cell counts of single cells and viability control skin biopsies and xenografts. As expected, the sequencing data showed a mix of mouse and human transcriptome profiles. However, after optimization, and unlike the mouse cells, all human cells presented as low-quality reads. We attribute this to preferential dissociation of mouse tissue and dead cells in the grafts.

Conclusion Here we present an advisory on tissue dissociation of xenografts for single cell applications. Dissociating both murine and human cells from a xenograft is required for successful processing in the scRNA-seq pipeline. However, we show that both tissues require different dissociation protocols. scRNA-seq from human xenografts may only be possible after careful selection of xenograft tissue only.

P21 – Anne-Lise Strandmoe

Deep phenotyping of circulating immune cell population in Pemphigus Vulgaris

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Background Pemphigus vulgaris (PV) is a potential fatal blistering disease characterized by intraepidermal loss of adhesion of the skin and mucosa due to the presence of autoreactive antibodies (auto-Abs) against the desmosomal cadherins; desmoglein (DSG) 1 and/or DSG3. Although the critical contribution of pathogenic autoantibodies is well accepted, the exact mechanism, functionality and immune interactions resulting in disease onset remain to be elucidated. Secondly, there remains a gap of knowledge regarding relapses after Rituximab (RTX) treatment.

Objective To gain deeper insight into the distribution and phenotype of major immune cell subsets in the peripheral blood of PV patients at active disease. Additionally, to acquire a deeper understanding of the circulating cytokines, chemokines and other signalling factors of PV patients at active disease and remission after RTX treatment.

Methods PBMC samples from 34 PV patients at active disease were analyzed using an established 40 marker full-spectrum flow cytometry panel for deep immunophenotyping covering all major immune cell subsets in human peripheral blood (OMIP-069)1. Additionally, we analyzed serum from 34 PV patients at active disease and 9 patients in remission after RTX treatment using a Luminex® panel of 46 analytes.

Results The data analysis is ongoing (Omiq.ai) and (preliminary) results will be presented at the NVED conference.

P22 – Coco Dekkers

Dupilumab-associated ocular surface disease in atopic dermatitis patients: clinical characteristics and pathogenesis

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Background Ocular surface disease (OSD) is frequently reported as adverse event during dupilumab treatment in atopic dermatitis (AD) patients.

Objective To investigate severity and frequency of dupilumab-associated ocular surface disease (DAOSD), the effect of ophthalmic therapies, and to learn more about the effect of dupilumab on conjunctival goblet cells (GC). In addition, conjunctival inflammation of (DA)OSD was examined by tear fluid biomarker analysis.

Methods This prospective study included moderate-to-severe AD patients who were examined by a dermatologist and an ophthalmologist both before and during dupilumab treatment (baseline, week 4, week 28). (DA)OSD severity was assessed by the Utrecht Ophthalmic Inflammatory and Allergic disease (UTOPIA) score. Conjunctival impression cytology was conducted to measure conjunctival GCs and additionally analyzed by flow cytometry. Tear fluid biomarkers were analyzed in a pilot study.

Results At baseline, OSD was seen in 91.3% (n=63/69) patients. DAOSD was observed in 28.9% (n=20/69) patients, in whom the number of GCs remained stable and Mucin 5AC (MUC5AC) expression decreased at onset of DAOSD compared with baseline. At week 28, 14.5% (n=10/69) of the patients had DAOSD. Of the 85.5% (n=59/69) patients without DAOSD or with controlled DAOSD at week 28, 40.7% (n=24/59) patients used anti-inflammatory ophthalmic treatment. No differences in Th1- or Th17-associated tear fluid biomarker levels were observed during dupilumab treatment (n=16).

Conclusion Moderate-to-severe AD patients often have OSD before starting dupilumab. Early ophthalmic treatment improves the severity of DAOSD during dupilumab treatment. The decrease in MUC5AC suggests an impairment of the GC function by dupilumab treatment.

P23 – Reineke Soegiharto

Epidemiology and management of chronic urticaria in primary care

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Background Management of chronic urticaria (CU) is widely studied in secondary and tertiary care populations, thus mostly in patients with difficult to treat disease. In the Netherlands, most CU patients are treated by the general practitioner (GP), the gatekeeper of the healthcare system. Data regarding management of CU in primary care is limited.

Objective To obtain insight in epidemiology, healthcare use and management of CU in primary care in the Netherlands.

Methods Patient data was retrieved from the Julius General Practitioners Network database, containing data from approximately 200 GPs and 450.000 patients in the region of Utrecht. Inclusion criteria: ICPC code S89 (urticaria); two visits for urticaria ≤180 days apart or >2 visits ≥ 28 days apart (to exclude acute urticaria); last GP contact due to urticaria between January 2010 and December 2019. Demographics, medication, follow-up duration and number of contacts were collected.

Results In total 4347 CU patients (66% female; mean age 33 years) were identified resulting in an estimated prevalence of 0.3-0.8%. Patients had a median of 4 (min-max: 2-307) GP contacts over a median follow-up duration of 19 (min-max: 1-370) months. During CU follow-up, 82% received CU treatment: mostly (91%) H1-antihistamine prescriptions with higher than standard dose in 14%. Systemic corticosteroids were prescribed in 7% of patients.

Conclusion In this Dutch primary care population we observed a CU prevalence of 0.3-0.8%. Urticaria related healthcare use consists of 4 GP contacts within 19 months. While antihistamines were often prescribed, following national guidelines, 7% of patients received systemic corticosteroids.