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MEETING REPORT

46th annual congress of the "Arbeitsgemeinschaft Dermatologische Forschung" in Munich, Germany

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1 | INTRODUCTION

The 46th annual congress of the "Arbeitsgemeinschaft Dermatologische Forschung" (ADF) was held in Munich from 14 March 2019 to 16 March 2019, organized by the local team of the TU Munich under the direction of Tilo Biedermann and Kilian Eyerich. With almost 500 participants and over 200 scientific contributions, the ADF was very pleased with the scientific excellence, with the well-balanced mixture of established researchers and aspiring new talents, as well as with the popularity of this conference.

It has become a wonderful tradition that the local organizers may leave their mark on the conference by implementing a workshop on the first day of the meeting, inviting exclusively speakers of their choice. This workshop in Munich, titled "Extending the borders of immunity," featured excellent presentations by Vasilis Ntziachristos (Neuherberg, Germany) on non-invasive observational techniques of skin pathophysiology, Jürgen Ruland (Munich, Germany) on immune sensing and immune metabolism, Mathias Heikenwälder (Heidelberg, Germany) on chronic inflammation, tissue damage, and tumorigenesis, and last but not least Stephan Herzig (Neuherberg, Germany) on tumor-host interactions and systemic energy homoeostasis.

As successfully established during the last years, the ADF annual conference features five major lectures which are embedded into presentations of the 36 best research projects selected from the participants' abstract submission by blinded peer review. Of note, this conference is designed with an exclusive single-track program without any overlapping events and differing locations to ensure a comprehensive, diverse, and yet inspiring content to incorporate all fields of dermatological research. The major lectures comprise the Guenter Goerz Memorial Lecture, the Guest Lecture, and three "Quo vadis" lectures. This year's prestigious Guenter Goerz Memorial Lecture was held by Nikolaus Romani (Innsbruck, Austria) elaborating beautifully on his research on Langerhans cells. Jacqueline Lees (Cambridge, USA) was invited for the Guest Lecture,

Prof. Dr. T. Biedermann Prof. Dr. K. Eyerich

presenting her impressive findings on differences between uveal and cutaneous melanoma. The three excellent "Quo vadis" lectures were given by Christoph Ellebrecht (Philadelphia, USA) on CAR T-cell therapy, Alexander Rösch (Essen, Germany) on overcoming hierarchies in tumor plasticity, and Georg Stary (Vienna, Austria) on tissue-resident T cells.

After 4 years of enthusiastic and tireless work as members of the ADF board, Ralf Ludwig, Kilian Eyerich and Karin Loser were seen off in style after receiving appropriate presents and a long applause from the ADF members. For their replacement, the members elected Mario Fabri, Georg Stary and Timo Buhl as incoming board members during the general assembly. According to the ADF statute, Evelyn Gaffal, Rüdiger Eming and Christoffer Gebhardt will continue their successful service as current board members for another 2 years.

Thanks to the generous support of our sponsors, the ADF was able to award various scientists and their successful work during this conference. The Paul Langerhans Prize was shared between Stefanie Eyerich (Munich, Germany) and Michael Hölzel (Bonn, Germany), sponsored by Celgene. Elsa Neubert (Göttingen, Germany) received the Egon Macher Prize for young scientists, sponsored by AbbVie. The Atopic Dermatitis Award was granted to Elke Rodriguez (Kiel, Germany), sponsored by Sanofi. Alexander Thiem (Würzburg, Germany) and Corinna Kosnopfel (Tübingen, Germany) were honoured with the Dermato-Oncology Junior Award, sponsored by Erwin + Irmgard-Egner Stiftung. The ADF/ECARF Award for Allergy Research was split between Denise Rauer (Augsburg, Germany) and Leonhard von Meyenn (Bern, Switzerland), sponsored by the European Centre for Allergy Research Foundation. For these aforementioned awards, the scientists had to apply in advance, and different juries were challenged to select the best scientist for the awards.

In addition to these mayor prizes with individual juries, the ADF was also able to grant several smaller awards to the presenters at the conference. These awardees were selected during poster-walks and presentations in Munich. Six Translational Research Prizes and twelve Poster Prizes were selected, mostly sponsored by Novartis and Almirall, and with help from Lilly, BioLegend, Dr. August Wolff, head&shoulders and Sanofi.

The ADF is very thankful for all the work and efforts which made this year's conference in Munich such a success. Besides scientists and staff, such a high-level scientific meeting would be impossible without the support of all companies which contributed to the annual conference through their generous support as "ADF supporting members," prize sponsors and/or exhibitors on-site. For further information and impressions of the Munich conference, please visit the ADF website (http://www.adf-online.de). Although the Munich conference has been wrapped up only recently, planning for next year's conference is already underway with selection and confirmation of the first speakers. The 47th ADF annual conference will be held from 11 March 2020 to 14 March 2020. The ADF board and the local organization team spearheaded by Kamran Ghoreschi and Marcus Maurer are looking forward to welcome you in Berlin.

2 | FIRST PAUL-LANGERHANS AWARD (S. EYERICH)

My scientific passion lies in understanding the pathology of atopic eczema and the improvement of diagnosis and therapy. With this given frame, our research studies in principle follow two paths—basic research on T-cell function and their communication with the epithelial barrier and translational research on the identification of disease biomarkers and targets for more efficient treatment regimens for atopic eczema.

The passion for T helper cells already evolved during my PhD thesis where we could show that a complete loss of Th17 cells is the immunological cause for chronic mucocutaneous candidiasis, a disease that is characterized by recurrent and/or chronic infection of epithelial barriers with the yeast Candida albicans. Motivated by this effect that a loss of a certain T cell subset can have, we were then looking for de-regulated T cell responses in atopic eczema besides the already known Th2 deviation. Here, we could describe a new subset of T helper cells, the Th22 cell that is highly enriched in inflamed skin and lung and regulates epithelial barrier integrity by initiation of epithelial host defense mechanisms and tissue repair after injury. We could further show that the functions of the name-giving cytokine, IL-22, are not per se protective but highly depend on the local cytokine microenvironment—presence of IFN-γ, for example, inhibits the tissue repair capability of Th22 cells, and *vice versa*, IL-22

reduces the pro-inflammatory capacity of IFN-γ. Exaggerated repair response mediated by IL-22 can, however, also turn pathologic as exemplary shown in psoriatic plaques that contain high numbers of Th22 cells and secreted IL-22.

T-helper cells are very communicative cells as they activate or modulate response profiles of epithelial cells by secretion of cytokines. But this communication is not uni-directional as also epithelial cells can modulate the function of T cells and thereby shape immune responses. Whereas keratinocytes are known modulators of immune responses, we could now show that also sebocytes take over an active role in tissue protection and inflammation. These stationary cells of the sebaceous gland are able to drive differentiation of naive T cells towards the Th17 phenotype and sebocyte-derived lipids induce alternatively activated macrophages with an inhibited secretion of proinflammatory cytokines and thereby act as immunemodulators before and during inflammation. These bi-directional interactions are highly fascinating and open new possibilities to influence epithelial mediated pathologies therapeutically.

Enhancing the diagnosis and treatment options for atopic eczema is one of our main goals driving many different research projects. In this area and with help of patients suffering from psoriasis and atopic eczema at the same time, we could develop a molecular diagnostic tool that is able to discriminate between psoriasis and atopic eczema by determining the expression levels of Nos2 and CCL27 in lesional vs non-lesional skin. This disease classifier is especially helpful for patients that are affected by inflammation at, for example, hand, foot, scalp where gold standards diagnostics often fail or lead to mis-diagnosis. The classifier has been patented and is currently developed for clinical routine diagnostic on histological sections in a fully automated lab-on-a-chip system.

With our unique cohort of double affected patients, we address another important task concerning atopic eczema—new therapeutic options. Even in times of Dupilumab, topical corticosteroids often represent the treatment of choice. Currently, we are expanding the existing data set and identify relevant and atopic eczema specific disease pathways that can be targeted via antibodies or small molecules not at the end of the pathogenic cascade, but right at the beginning of it. The first candidates are currently tested in our therapeutic in vitro pipeline including CRISPR/Cas9 over-expression or silencing of genes in human primary cells, cellular co-culture systems and 3D skin models. So we are facing exciting times in research and look forward to the future where atopic eczema patients are treated in a personalized manner.

3 | SECOND PAUL-LANGERHANS AWARD (M. HÖLZEL)

For more than 20 years, I am interested in the molecular mechanisms of tumorigenesis and resistance to cancer therapy. Malignant melanoma is a paradigm disease for the development of new targeted cancer therapeutics. Clinical advances in other cancer types have clearly benefited from findings and novel treatment concepts pioneered by the melanoma field. For this reason, melanoma became the focus of my group, as studying and understanding this skin malignancy requires an inspiring interdisciplinary effort to combine the knowledge from different research areas such as immunology, tumor biology and embryonal development.

Great advances have been achieved in the clinic for the treatment of patients with advanced stage melanoma. The two most prominent examples are small molecule inhibitors of oncogenic MAPK (mitogen-activated protein kinases) signalling and immune checkpoint inhibitors that reinvigorate anti-tumor immunity. Despite these clinical breakthroughs, primary and acquired resistance remain major challenges limiting therapeutic benefit. Given rapid development of sequencing technologies, the global melanoma research community accumulated information on genomic aberrations found in melanoma including also candidate drivers of therapy resistance. Whereas hardwired genomic changes can be effectively traced by next-generation sequencing approaches and appropriate algorithms, dynamic and reversible epigenetic changes are more difficult to capture, but are equally important for melanoma cells to cope with therapeutic selection pressure and stressful microenvironmental conditions. This adaptation is also termed phenotype switching or phenotypic plasticity and it became clear that melanoma is an exquisite example to study this phenomenon.

Over the past years, my research group and many instrumental collaborators have put a lot of effort to disentangle the molecular networks that are involved in melanoma phenotype switching. For example, we identified an antagonism between the melanocyte lineage transcription factor MITF and the stress signalling hub c-JUN/AP-1 that governs the response of melanoma cells to inflammatory signals. Currently, we are interested in developing functional genomics tools to decode and record the dynamics of melanoma phenotype switching. We expect that fuelling appropriate mathematical models with detailed experimental data will provide us with novel concepts about the underlying principles.

Furthermore, we have a particular interest how melanoma cells crosstalk with the immune system. In the context of immunotherapy, we recently found that the inflamed tumor microenvironment induces T cell suppressive properties in reactively infiltrating neutrophils limiting the efficacy of different immunotherapies. As reactive neutrophil infiltration could be reduced by inhibitors of the receptor tyrosine kinase c-MET, our findings support combinations of c-MET inhibitors with immune checkpoint blockade, which are currently under clinical investigation in melanoma and other cancers. In summary, the melanoma field has witnessed fascinating developments in the recent years and it is a joyful and rewarding task to get young scientists excited about this disease in order to support the next generation that moves the field forward to the benefit of many patients.

4 | EGON-MACHER AWARD (E. NEUBERT)

Neutrophil granulocytes are able to release a fibril network consisting of decondensed neutrophil DNA and antimicrobial peptides,

termed neutrophil extracellular traps (NETs), to catch and immobilize pathogens. The dysregulation of NET formation is implicated in various pathological conditions including a large range of infectious and malignant diseases. Therefore, it is highly important to understand the regulation of this evolutionarily conserved innate immune response. While the underlying signalling has been the focus of numerous studies, the biophysical processes governing NET formation still remained enigmatic. Therefore, we focused on identifying the exact driving forces, which allow the release of decondensed neutrophilic chromatin through the rupture of the cells' plasma membrane.

In our study "Chromatin swelling drives neutrophil extracellular trap release," published in 09/2018 in the journal *Nature Communications*, we analysed the regulation of NET formation in detail on the single-cell level, mainly by employing various life-cell imaging approaches. We could show that two fundamentally different driving forces govern NET formation. Enzymatic processes are required at the beginning to initiate the morphological changes within the cell. However, the progression of NET formation as well as the final NET release is mostly driven by the entropic swelling of the expanding chromatin. Indeed, the onset of chromatin expansion, which coincides with the fragmentation of the nuclear envelope, represents a *point of no return*. From this point on, the formation of NETs cannot be pharmacologically inhibited any longer. Interestingly, the position of the final rupture point induced by chromatin swelling is biomechanically predetermined by the initial position of the nucleus as well as membrane dynamics directly before NET release.

In conclusion, we could show that the complex behaviour of cells does not only depend on active enzymatic signalling. It can also be regulated by the cells' material properties, such as the entropic swelling of the expanding chromatin network in the case of NETosis. This observation is, on the one hand, particularly relevant for the development of new pharmaceutical strategies to interfere with NET formation. On the other hand, the concept of "active matter" (in this case the expanding chromatin) as a key player in biological processes greatly broadens our understanding of the regulation of cellular behaviour.

5 | FIRST DERMATO-ONCOLOGY AWARD (C. KOSNOPFEL)

5.1 | Inhibition of RSK family members can effectively target malignant melanoma cells with MAPK pathway hyperactivation

The MAPK signalling pathway is frequently hyperactivated in malignant melanoma and plays a central role in tumor cell proliferation and survival. Accordingly, its inhibition has proven to be an efficient treatment option for melanomas harbouring BRAF mutations. However, there is still a considerable need for effective targeted therapies for other melanoma subgroups with constitutive MAPK activation, such as RAS- and NF-1-mutated tumors, as well as for therapeutic options targeting MAPK pathway inhibitor resistant BRAF-mutated melanomas, which commonly exhibit a striking reactivation of this pathway.

The p90 ribosomal S6 kinases (RSKs) are directly activated by ERK1/2 and represent central effectors of the MAPK signalling regulating cell cycle progression and survival. Based on that, our work "Inhibition of RSK family members can effectively target malignant melanoma cells with MAPK pathway hyperactivation" assessed a potential functional role of the p90 ribosomal S6 kinases and their inhibition in different MAPK pathway-driven genetic melanoma subgroups.

Indeed, we revealed an increased RSK activity going along with hyperactivation of the MAPK pathway not only in BRAF but also in RAS- and NF-1-mutated melanoma cells. Interestingly, RSK-specific small molecule inhibitors were able to effectively target those cells, particularly when applied chronically in three-dimensional growth systems.

In line with a pronounced reactivation of the MAPK pathway in the case of MAPK pathway inhibitor resistance, we observed a further increase in RSK activity in BRAF-mutated melanoma cells with acquired MAPK pathway inhibitor resistance both in an in vitro model system and in tumor biopsies from stage IV melanoma patients which progressed under MAPK pathway inhibitor therapy. Accordingly, MAPK pathway inhibitor resistant melanoma cells proved to be particularly sensitive to RSK inhibition, leading to cell cycle arrest and eventually apoptosis induction.

Overall, these data indicate a potential general usefulness of the p90 ribosomal S6 kinase family members as prospective targets in malignant melanoma with hyperactivated MAPK signalling.

6 | SECOND DERMATO-ONCOLOGY AWARD (A. THIEM)

6.1 | IFN-gamma-induced PD-L1 expression in melanoma depends on p53 expression

Immune checkpoint inhibition and in particular anti-PD-1 immunotherapy have revolutionized the treatment of advanced melanoma. In this regard, higher tumoral PD-L1 protein (gene name: *CD274*) expression is associated with better clinical response and increased survival to anti-PD-1 therapy. Furthermore, there is increasing evidence that tumor suppressor proteins are involved in immune regulation and are capable of modulating the expression of immune checkpoint proteins. In our project, we determined the role of p53 protein (gene name: *TP53*) in the regulation of PD-L1 expression in melanoma.

Initially, we performed immunohistochemistry on tumors with known *TP53* status and found *TP53*-mutated tumors to be more often PD-L1 positive. Analysis of publicly available mRNA and protein expression data from the cancer genome/proteome atlas (TCGA/TCPA) revealed that *TP53*-mutated tumors also express more *CD274* mRNA. Interestingly, lack of correlation between p53 target gene expression and *CD274* mRNA, but a positive correlation

between p53 and PD-L1 protein suggests a non-transcriptional mode of action of p53.

To further explore the role of p53 in the regulation of PD-L1, we performed cell line experiments upon p53 knockdown in wildtype, *TP53*-mutated or JAK2-overexpressing melanoma cells or in cells, in which p53 was rendered transcriptionally inactive by CRISPR/Cas9. Constitutive and IFN-ɣ-induced PD-L1 expression was assessed by immunoblot or flow cytometry. Immunoblot was also applied to analyse the impact on the IFN-ɣ signalling (JAK-STAT)-pathway. Cell line experiments revealed a diminished IFN-ɣ-induced PD-L1 expression upon p53 knockdown in both wildtype and *TP53*-mutated melanoma cells, which was not the case when p53 wildtype protein was rendered transcriptionally inactive. The impaired PD-L1-inducibility was associated with a reduced JAK2 expression in the cells and was almost abrogated by JAK2 overexpression.

In summary, we demonstrated that p53 plays an important positive role for IFN-ɣ-induced PD-L1 expression in melanoma cells by supporting JAK2 expression in a transcription independent manner. Future studies should address, whether p53 expression levels might influence responses to anti-PD-1 immunotherapy.

7 | ECARF AWARD (L. VON MEYENN)

7.1 | Human "TH9" cells are a subpopulation of PPARγ+ TH2 cells

T helper (TH) cell subsets are crucial mediators of adaptive immune responses. To respond to the various infectious and non-infectious challenges, they have evolved into specialized subsets. TH cell subsets are defined by the cytokine environment under which they differentiate, the transcription factors that genetically regulate their phenotype, the chemokine receptors that define their migratory potential, and the cytokines they secret to exert their specialized functions. While TH1, TH2 and TH17 cells are well-defined T helper cell lineages in humans, the existence of an IL-9-producing "TH9" lineage remains debated. Given the functional importance of "TH9" cells in murine models of allergic inflammation and tumor immunity, we here set out to better understand the identity of human IL-9-producing TH cells by first characterizing their subset-defining properties. We found that IL-9-producing TH cells are better described as a subpopulation of TH2 cells that express IL-9 transiently post activation, rather than as a bona fide TH cell lineage. This is based on our findings that IL-9-producing TH cells show key TH2-lineage-defining properties: IL-9-producing TH cells express TH2-lineage-defining cytokines (IL-5, IL-13), chemokine receptors (CCR4+/CCR8+), and transcription factors (GATA3) when analysed irrespective of activation status.

Next, we investigated the transcriptional program that differentiates IL-9+ TH2 cells from "conventional" TH2 cells that lack IL-9 expression. To this end, we performed transcriptional profiling before and after activation of different human TH cell subsets. We found that IL-9+ TH2 cells specifically express high levels of the transcription factor PPARγ. Accordingly, PPARγ was strongly induced in naive TH cells by priming with IL-4 and TGF-β ("TH9" priming), just as IL-9 itself. Functional importance of PPARγ for IL-9 expression was confirmed by pharmacological antagonism with GW9662 or gene silencing of PPARγ by siRNA. PPARγ inhibition reduced IL-9 production in TH2 cells while leaving production of other cytokines in TH2, TH1 and TH17 cells largely unaffected.

In human skin disease, we found high numbers of IL-9+ TH2 cells in acute but not chronic allergic skin inflammation and these numbers correlated with the presence of PPARγ+ cells. Correspondingly, antagonism of PPARγ in T cells isolated from acute allergic contact dermatitis resulted in specific downregulation of IL-9. Taken together, these findings suggest PPARγ as novel regulator of IL-9 in TH2 cells and identify TGF-β as an important factor inducing PPARγ in human TH2 cells. Our findings in humans are in line with recent findings in murine models of allergy and parasite infection where PPARγ emerged as a driver of pathogenic TH2 inflammation.

8 | ATOPIC DERMATITIS AWARD (E. RODRÍGUEZ)

8.1 | Exome-wide association study reveals DOK2 as novel atopic dermatitis susceptibility gene harbouring low frequency risk variants

Atopic dermatitis (AD) is a common chronic-inflammatory skin disease which is based on the complex interplay of multiple genetic and non-genetic factors. Its genetic heritability has been estimated to be about 90% in Europeans, and the discovery of the disease's causal genes is a crucial step in finding effective treatment for AD. Since 2009 genome-wide association studies (GWAS) for AD have identified more than 30 susceptibility loci, but the vast majority of the reported susceptibility variants are common (minor allele frequency (MAF) ≥ 5%), located in non-coding genomic regions, have small effect sizes (odds ratio (OR) < 1.15) and an unclear functional significance.

To systematically evaluate the contribution of genetic variation, particularly protein-altering variants of low (1% ≤ MAF < 5%) or rare frequency (MAF < 1%), to the genetic architecture of AD on the exome-wide scale, we profiled 1913 AD patients and 14 295 controls in two German cohorts using the Illumina HumanExomeBeadchip (exome chip), which covers approximately 88% of low and rare frequency coding variants present in Europeans, followed by replication in an independent set of 4379 AD cases and 11 724 controls of European descent.

We identified the two novel susceptibility genes *Docking protein 2* (*DOK2*) and *CD200 Receptor 1* (*CD200R1*) harbouring AD-associated missense variants of common, low and rare frequency. Protein sequence and in silico structural protein domain analyses suggest that these missense variants jointly affect important phosphorylation sites in DOK2 and CD200R1 that are necessary for immune receptor signalling in T helper 2 cells. Multiomics-based network interaction analysis combined with whole transcriptome data on

lesional, non-lesional and healthy skin further revealed *DOK2* as a central hub in our AD core disease network interacting amongst others with *CD200R1* and established AD risk genes such as *IL6R* and *STAT3*. 22 out of the 30 identified AD core genes are significantly up or downregulated in lesional AD skin and interact directly with *DOK2*, thus indicating the biological importance of *DOK2* for AD. Moreover, tissue-specific gene expression enrichment analysis within 53 tissue types from GTEx showed that exome chip variants associated with AD cumulatively have a stronger effect on skin tissue gene expression than common variants from large AD consortium GWAS.

We conclude that signalling through CD200/CD200R1/DOK2 represents an important new regulatory pathway in AD and our discoveries highlight the potential role of low and rare coding variants acting independently of common GWAS variants in AD aetiology.