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MEETING ABSTRACTS

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Correspondence

Intrinsic Activity
c/o Otto Loewi Research Centre (Chair of Pharmacology)
Medical University of Graz
Universitätsplatz 4
8010 Graz, Austria
E-mail: info@intrinsicactivity.org

Website: www.IntrinsicActivity.org
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Austrian Pharmacological Society
c/o Institute of Pharmacology
Centre for Physiology and Pharmacology
Medical University of Vienna
Währinger Straße 13a
1090 Wien, Austria
E-mail: office@aphar.at

Conference poster: thomasgraz.net

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PUBLISHED MEETING ABSTRACTS

Keynote Lectures

A1.1

Pharmacometrics – Opportunities in pharmacological research and clinical practice

Iris Katja MINICHMAYR*

Department of Pharmacy, Uppsala University, Uppsala, Sweden
*E-mail: *iris.minichmayr@farmaci.uu.se*

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Pharmacometrics represents a multi-disciplinary research field that describes and quantifies (patho-)physiological processes in a system (*e.g.* a patient) and its interactions with drugs using mathematical and statistical models. These models are frequently developed to characterise time courses of drug exposure (pharmacokinetics), drug effects (pharmacodynamics) and/or disease progression, and enable conclusions on favourable or unfavourable drug responses. Thereby, data from different sources (*e.g. in vitro* data and clinical data) can be analysed and jointly integrated.

Since its emergence in the 1970s, pharmacometrics has experienced a remarkable growth. Pharmacometrics has revolutionised rational decision-making in pharmaceutical industry across the value chain ('model-informed drug development'), but also in pharmacotherapy and patient care to tailor dosing strategies, improve therapeutic response and prevent toxicity ('model-informed precision dosing'). Apart from that, regulatory agencies like the U.S. Food and Drug Administration and the European Medicines Agency have strongly supported pharmacometric analyses and their inclusion in regulatory submission dossiers and even drug labels.

Nonlinear mixed-effects (NLME) models, often referred to as 'population (pharmacokinetic or pharmacodynamic) models', enable to simultaneously analyse data (*e.g.* concentration–time profiles) of an entire population, while still not ignoring differences between individuals (*e.g.* patients). On this basis, the typical representative of the data (*i.e.* the 'typical patient' in the population) can be described, together with different sources of variability (*e.g.* between-patient variability). Importantly, potential causes of this variability, *e.g.* patient- or disease-related factors ('covariates') like body weight, renal function or genetic polymorphisms, can be identified, which constitute the basis for dosing individualisation.

Once successfully developed and evaluated, pharmacometric models can be used for simulations to answer 'what-if' questions. For example, study scenarios not covered by the raw data (*e.g.* new dosing regimens) as well as extrapolations to other study populations (*e.g.* from preclinical species to humans), can be investigated. Simulations, specifically clinical trial simulations, can also serve to inform the design of future clinical trials in a resource-saving manner or to derive hypotheses for further studies.

This keynote lecture aims to outline the basic concepts of pharmacometrics and diverse applications and opportunities of this research area in preclinical and clinical research. Examples of past accomplishments of pharmacometrics will span different therapeutic areas with a focus on, but not limited to, antibiotic research and continuous data (*i.e.* time courses of observations). Last, an outlook of pharmacometric tools to individualise dosing in clinical practice and its role in therapeutic drug management will be given, together with further promising developments in this rapidly evolving field.

Keywords: pharmacometrics – population pharmacokinetic models – population pharmacokinetic/pharmacodynamic models – PK/PD – modelling and simulation – model-informed precision dosing

A1.2

From hypersensitivity to leukemia: insights into function and pharmacology of cation channels

Susanna ZIERLER[1,2,*]

[1]Institute of Pharmacology, Medical Faculty, Johannes Kepler University Linz, Austria;[2]Walther Straub Institute of Pharmacology and Toxicology, Faculty of Medicine, Ludwig Maximilian University Munich, Germany
*E-mail: *susanna.zierler@jku.at*

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While the immune system protects our body against numerous pathogens, it must not overshoot. Otherwise, pro-inflammatory diseases or anaphylactic reactions may develop. Thus, several mechanisms are in place to ensure an appropriate immune response at the right time. Immune cells themselves are tightly controlled by their intricate cellular cation homeostasis and signalling.

The ubiquitously expressed organellar two-pore channel, TPC1, for instance, has been suggested to be involved in spatial Ca^{2+} homeostasis in different cell types. We recently demonstrated a crucial role of TPC1 in controlling organellar Ca^{2+} homeostasis. *Tpc1*-deficient (*Tpc1*$^{-/-}$) mice develop enhanced systemic anaphylaxis upon crosslinking of IgE receptors. Genetic deletion or pharmacologic inhibition of TPC1 enhances mast cell degranulation and histamine release due to accelerated Ca^{2+} liberation mainly from the endoplasmic reticulum (ER). Accordingly, pharmacologic activation of TPC1 ameliorates mast cell degranulation, highlighting TPC1 as a potential drug target against allergic hypersensitivity [1].

Similarly, we have linked the dual-function transient-receptor-potential protein, TRPM7, combining a Ca^{2+}- and Mg^{2+}-permeable channel with a serine/threonine kinase, to immune system homeostasis. Exploring the role of the TRPM7 kinase moiety in mast cells,

Edited by: Thomas Griesbacher (Austrian Pharmacological Society APHAR, Otto Loewi Research Centre, Section of Pharmacology, Medical University of Graz, Austria; *thomas.griesbacher@medunigraz.at*)

we found that its genetic disruption prompts altered susceptibility to allergic reactions in mice [2]. Using a homozygous kinase-dead mouse model with a single point mutation at the active site of the kinase, *Trpm7*^{K1646R}, we demonstrated that its activity controls TGF-β-induced CD103 expression, intestinal T cell colonization as well as proinflammatory T_H17 cell differentiation, but is dispensable for anti-inflammatory, regulatory T cell differentiation. Notably, we identified SMAD2 as novel substrate of the TRPM7 kinase. Ultimately, genetic disruption of the TRPM7 kinase activity prevents the development of acute graft-*versus*-host-disease in an established mouse model [3]. To date, specific pharmacologic modulators of TRPM7 channel or kinase are limited. Previously, we were able to discover the natural compound, waixenicin A, isolated from the Hawaiian soft coral, as first potent and selective TRPM7 channel inhibitor [4]. Currently, we are screening for potent TRPM7 kinase inhibitors utilizing *in silico* techniques. Our first translational results imply that TRPM7 is also important for the differentiation of human T cells as well as for the activation and proliferation of chronic leukemia cells. Therefore, TRPM7 channel and kinase may represent valid pharmacological targets for the treatment of pro-inflammatory diseases and chronic leukemia.

Keywords: immune system – cation channels – calcium – homeostasis – T cell differentiation – mast cell reactivity – TPC1 – TRPM7

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Short Presentations

A2.1

K_V7 channels as targets for paracetamol

Isabella SALZER[1,*], Sutirtha RAY[1], Jan-Luca STAMPF[1], Oliver KUDLACEK[2], Jae-Won YANG[2], Stefan BOEHM[1]

[1]*Division of Neurophysiology and Neuropharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Austria;* [2]*Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Austria*

*E-mail: *isabella.salzer@meduniwien.ac.at*

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Background: Voltage-gated K_V7 channels are expressed in a variety of excitable tissues such as the nervous or cardiovascular system. Neuronal K_V7 channels regulate excitability in neurons; consequently, reduced activity leads to states of hyperexcitability, like epileptic seizures or neuropathic pain. Both pathologies can be treated by increasing currents through these channels, for example via cysteine modification. Despite being used for over 60 years, the mechanism of action of paracetamol remains debated. Metabolites of paracetamol are suggested to mediate its analgesic effect. Most notably, the toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) was suggested to also mediate the therapeutic effect via cysteine modification of ion channels.

Methods: Currents through native neuronal and heterologously expressed K_V7 channels were recorded in the perforated voltage-clamp mode. Action potentials in primary first-order (dorsal root ganglion, DRG) and second-order (spinal dorsal horn, SDH) neurons of the pain pathway were recorded in the perforated current-clamp mode. Covalent NAPQI adducts were analyzed by mass-spectrometry analysis. K_V7 channel involvement *in vivo* was tested via inducing inflammation by s.c. injection of carrageenan (100 µl of 2% w/v) into the right hind paw of Sprague-Dawley rats. Nocifensive behavior was tested both with mechanical (using von-Frey filaments) and thermal (Hargreaves test) pain paradigms.

Results: Paracetamol administration (300 mg/kg; i.p.) increased withdrawal forces but not thermal withdrawal latencies. The latter of which could be prevented by co-administration of XE 991 (3 mg/kg, i.p.), a blocker of K_V7 channels. Application of NAPQI (1 µM) reduced the number of action potentials in primary cultures of rat DRG and SDH neurons. This effect could be prevented by application of the K_V7 channel blocker linopirdine (30 µM). NAPQI increased currents through K_V7 channels in both types of neurons but currents remained unaltered in presence of the parent compound paracetamol (100 µM). The current increase could be reproduced in heterologously expressed $K_V7.2$ channels. Application of NAPQI covalently modifies one out of a stretch of three cysteine residues in the S2–S3 linker region (C150–C152). Each cysteine residue can be modified, but only a single cysteine residue is modified at the same time. Alanine substitution of all three cysteine residues prevented K_V7 currents from increasing in response to NAPQI application. In addition, co-transfection of apo-calmodulin also prevented K_V7 currents from increasing in response to NAPQI application.

Discussion: Administration of paracetamol reduces mechanical hyperalgesia in rats but not thermal hyperalgesia. The former effect involves K_V7 channels. On a cellular level, this effect corresponds to reduced action-potential frequencies in first- and second-order neurons of the pain pathway in response to NAPQI application, which, again, involves K_V7 channels. Currents through K_V7 channels are increased in response to the paracetamol metabolite NAPQI, but neither to the parent compound nor to other metabolites. On a molecular level, this effect is mediated by covalent modification of one out of three subsequent cysteine residues in the S2–S3 linker

region. This region is known to mediate Ca^{2+}-dependent modulation of K_V7 channels via calmodulin. Indeed, the ability of calmodulin to sense Ca^{2+} seems to be involved in this process.

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Keywords: K_V7 channels – paracetamol – NAPQI

A2.2

The ferroptosis and MEK1/2 inhibitor U0126 improves functional outcome after intracerebral hemorrhage in mice independent of ERK1/2

Marietta ZILLE[1,2,3,*], Juan A. OSES-PRIETO[4], Sara R. SAVAGE[5], Saravanan S. KARUPPAGOUNDER[2,3], Yingxin CHEN[2,3], Amit KUMAR[2,3], John H. MORRIS[6], Karl A. SCHEIDT[7], Alma L. BURLINGAME[4], Rajiv R. RATAN[2,3]

[1]Department of Pharmaceutical Sciences, Division of Pharmacology and Toxicology, University of Vienna, Austria; [2]Burke Neurological Institute, White Plains, NY, USA; [3]Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York, NY, USA; [4]Department of Pharmaceutical Chemistry, University of California, San Francisco, CA, USA; [5]Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX, USA; [6]Resource on Biocomputing, Visualization, and Informatics, University of California, San Francisco, CA, USA; [7]Department of Chemistry, Center for Molecular Innovation and Drug Discovery, Northwestern University, Evanston, IL, USA

*E-mail: *marietta.zille@univie.ac.at*

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Background: Intracerebral hemorrhage (ICH) is a devastating neurological disease without effective treatment options. Neuronal cell death induced by the blood breakdown products hemoglobin and hemin after ICH is executed by regulated cell death mechanisms including ferroptosis, necroptosis, and autophagy. Ferroptosis is a caspase-independent form of regulated necrosis that is activated by oxidative stress induced by glutathione depletion, enzymatic derived reactive lipid species, and hemin. Recently, we identified the MAP kinase kinase 1/2 (MEK1/2) inhibitor U0126 as an anti-ferroptotic agent that abrogated neuronal cell death in experimental models of ICH *in vitro*. In this study, we evaluated hyperactivation of extracellular signaling kinase 1/2 (phospho-ERK1/2) as a target for ICH therapy.

Methods: We performed careful dose finding of U0126 and examined its effect on functional recovery after collagenase-induced ICH in mice. Further, we verified the molecular knockdown of phospho-ERK1/2 by overexpressing MAP kinase phosphatase (MKP) for its ability to protect neurons from ferroptosis after hemorrhagic stroke in addition to using chemically diverse inhibitors of MEK. In search for the mechanism of U0126, we performed an unbiased phosphoproteome analysis.

Results: We demonstrate that the ferroptosis and MEK1/2 inhibitor U0126 improves functional recovery after ICH in mice. Whereas ICH leads to chronic hyperactivation of ERK1/2, U0126 prevented hemin-induced ferroptosis independent of its ability to inhibit ERK1/2 signaling. In contrast to classical ferroptosis in neurons or cancer cells, chemically diverse inhibitors of MEK did not block hemin-induced ferroptosis, nor did the forced expression of the ERK-selective MAP kinase phosphatase 3. We further show that phospho-ERK1/2 accumulates in the cytoplasm and is therefore unable to induce MKP1 and MKP3, its negative regulators, leading to chronic hyperactivation. Remarkably, our unbiased phosphoproteome analysis revealed dramatic differences in phosphorylation induced by classical *vs.* ICH-induced ferroptosis and provides novel insights into the mechanism of U0126.

Discussion: Taken together, our data suggests that the anti-ferroptotic agent U0126 promotes functional recovery independent of its ability to abrogate the chronic hyperactivation of ERK1/2 after ICH. These studies define distinct subtypes of neuronal ferroptosis and provide a template on which to build a search for U0126's effects in a variant of neuronal ferroptosis.

Acknowledgements: This work was supported by grant DFG Zi 1613/1-1 to M.Z. from the German Research Foundation, funding from the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation and the Sperling Center for Hemorrhagic Stroke Recovery and NIH (grant P01 NIA AG014930, project 1) to R.R.R.

Keywords: brain hemorrhage – cell death – ERK – ferroptosis – MAP kinase – stroke

A2.3

A STAT5B–CD9 axis determines self-renewal in hematopoietic and leukemic stem cells

Sebastian KOLLMANN[1,*], Reinhard GRAUSENBURGER[1], Thorsten KLAMPFL[1], Michaela PRCHAL-MURPHY[1], Klavdija BASTL[1], Hanja PISA[2], Vanessa M. KNAB[1], Tania BRANDSTOETTER[1], Eszter DOMA[1], Wolfgang R. SPERR[3,4], Sabine LAGGER[5], Matthias FARLIK[6,7], Richard MORIGGL[8], Peter VALENT[3,4], Florian HALBRITTER[2], Karoline KOLLMANN[1], Gerwin HELLER[1,9], Barbara MAURER[1], Veronika SEXL[1]

[1]Institute of Pharmacology and Toxicology, University of Veterinary Medicine, Vienna, Austria; [2]St. Anna Children's Cancer Research Institute (CCRI), Vienna, Austria; [3]Division of Hematology and Hemostaseology, Department of Medicine I, Comprehensive Cancer Center, Medical University of Vienna, Austria; [4]Ludwig Boltzmann Institute for Hematology and Oncology, Medical University of Vienna, Austria; [5]Unit of Laboratory Animal Pathology, University of Veterinary Medicine, Vienna, Austria; [6]CeMM Research Center for Molecular Medicine, Austrian Academy of Sciences, Vienna, Austria; [7]Department of Dermatology, Medical University of Vienna, Austria; [8]Institute of Animal Breeding and Genetics, Unit for Functional Cancer Genomics, University of Veterinary Medicine, Vienna, Austria; [9]Clinical Division of Oncology, Department of Medicine I, Medical University of Vienna, Austria

*E-mail: *veronika.sexl@vetmeduni.ac.at*

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Background: The JAK/STAT5 signaling pathway is essential in hematopoiesis and leukemogenesis. Activating STAT5 mutations in leukemia have been found exclusively in STAT5B, and despite considerable efforts no mutations in STAT5A have been identified. This seems to contradict the widely held belief that the two have essentially homologous functions. We now resolve the paradox by providing the first proof that STAT5A and STAT5B have different roles in hematopoietic stem cells (HSCs). Like HSCs, leukemic stem cells (LSCs) have the ability to self-renew and represent a major therapeutic challenge because of their high drug resistance, by stem-cell-specific properties including slow cell division, enhanced drug efflux or increased DNA repair. Therefore, it is important to identify novel mechanisms to target LSCs to enhance the effectiveness of curative approaches. The LSC-dependent diseases chronic myeloid leukemia (CML), acute myeloid leukemia (AML) or myeloproliferative neoplasm (MPN) examples include BCR/ABL^{p210}, FLT3-ITD or $JAK2^{V617F}$, where disease development requires STAT5 signaling. It remains enigmatic why STAT5B and not STAT5A is mutated, and whether the oncogenes activate both.

Methods: We used a broad range of *in vivo*, *ex vivo* and *in vitro* experiments to characterize HSCs and LSCs. We performed single-cell RNA-Seq to identify novel STAT5A and STAT5B target genes in

HSCs, validated by ChIP-qPCR in murine hematopoietic progenitor cell lines. Finally, we translated our findings from various murine models to samples of human leukemia patients.
Results: We found a selective activation of STAT5B in HSCs and LSCs defining it as a key player in stem-cell quiescence and self-renewal, a function not shared by STAT5A. We generated single-cell RNA-Seq data to define a STAT5B-specific HSC signature and identified CD9 as a downstream target. Elevated CD9 levels are associated with a poor prognosis in AML patients. The elevated STAT5B/CD9 signaling in LSCs makes them sensitive to anti-CD9 antibodies, which induce differentiation and apoptosis.
Discussion: Our findings emphasize the importance of distinguishing between STAT5A and STAT5B. Our concept of selective STAT5B activation downstream of cytokine and oncogenic signaling paves the way for novel treatment approaches, here exemplified by CD9 blocking.
Acknowledgements: This work is supported by the Austrian Science Fund FWF-SFB 6107.
Keywords: STAT5A – STAT5B – hematopoietic stem cells – leukemic stem cells – CD9

A2.4

Triple-negative breast cancer cells rely on kinase-independent functions of CDK8 to evade NK-cell-mediated tumor surveillance

Vanessa M. Knab[1], Dagmar Gotthardt[1], Klara Klein[1], Reinhard Grausenburger[1], Gerwin Heller[2], Ingeborg Menzl[1], Daniela Prinz[1], Jana Trifinopoulos[1], Julia List[1], Daniela Fux[1], Agnieszka Witalisz-Siepracka[1], Veronika Sexl[1,*]

[1]Institute of Pharmacology and Toxicology, University of Veterinary Medicine, Vienna, Austria; [2]Division of Oncology, Department of Medicine I, Medical University of Vienna, Austria
*E-mail: *veronika.sexl@vetmeduni.ac.at*
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Background: Triple-negative breast cancer (TNBC) is an aggressive malignant disease that is responsible for approximately 15% of breast cancers. The standard of care relies on surgery and chemotherapy but the prognosis is poor and there is an urgent need for new therapeutic strategies. Recent *in silico* studies have revealed an inverse correlation between recurrence-free survival and the level of cyclin-dependent kinase 8 (CDK8) in breast cancer patients. CDK8 is known to have a role in natural killer (NK)-cell cytotoxicity, but its function in TNBC progression and immune-cell recognition or escape has not been investigated.
Methods: We used a murine model of orthotopic breast cancer to study the tumor-intrinsic role of CDK8 in TNBC. To shed new light onto the function of NK cells in the control of the primary tumor and of metastasis we additionally performed NK depletion experiments in our mouse models. RNA sequencing was carried out to highlight modulators and CDK8-dependent pathways.
Results: Knockdown of CDK8 in murine TNBC cells impairs tumor regrowth upon surgical removal and prevents metastasis formation *in vivo*. In the absence of CDK8, the epithelial-to-mesenchymal transition (EMT) is impaired and immune-mediated tumor-cell clearance is facilitated. *In vivo* experiments confirmed that CDK8 is a crucial regulator of NK-cell-mediated immune evasion in TNBC. Using a CDK8/CDK19 kinase inhibitor we failed to detect any effect of CDK8 on EMT transcription factors, suggesting a kinase-independent regulation. Differential gene expression shows that CDK8 is involved in regulating the checkpoint inhibitor programmed death-ligand 1 (PD-L1). The CDK8–PD-L1 axis is found in mouse TNBC cells and is supported by a dataset of human TNBC patients where the levels of PD-L1 and CDK8 expression positively correlate.
Discussion: We identified CDK8 as a critical regulator of tumorigenesis and describe it as a novel immune checkpoint. It controls metastatic properties of TNBC cells and drives NK-cell immune evasion. Our data link CDK8 to PD-L1 expression and provide a rationale for investigating the possibility of CDK8-directed therapy for TNBC.
Acknowledgements: This work was supported by the Austrian Science Fund FWF (P27248-B28, P28571-B21, PhD program "Inflammation and Immunity" W1212, FWF doc.funds DOC32-B28, and the Stadt Wien Kultur MA7 Grant FA18020013.
Keywords: triple-negative breast cancer – NK cells – immune evasion – tumor immune surveillance – epithelial-to-mesenchymal transition – metastasis

A2.5

Myeloperoxidase positively regulates non-small-cell lung cancer development

Paulina Valadez Cosmes, Nejra Ćosić, Kathrin Maitz, Melanie Kienzl, Sofia Raftopoulou, Ákos Heinemann, Julia Kargl*

Division of Pharmacology, Otto Loewi Research Center, Medical University of Graz, Austria
*E-mail: *julia.kargl@medunigraz.at*
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Background: Myeloperoxidase (MPO) is predominantly expressed by neutrophils and expressed in their primary granules. It is a heme peroxidase that catalyses the reaction of hydrogen peroxide with halides (Cl^- and Br^-) and pseudo-halide (SCN^-) to generate powerful oxidants that can have important biological effects by modifying proteins, lipids and DNA. Neutrophils are a prevalent immune-cell population in the tumor microenvironment (TME) of non-small-cell lung cancer (NSCLC). In this context, some of the neutrophil granule components have been proposed to contribute to tumor proliferation, angiogenesis and metastasis. Moreover, an impaired function and proliferation of lymphocytes has been linked to the action of neutrophils in the TME. However, few is known regarding the effects of MPO on NSCLC development.
Methods: *In vivo*: MPO knockout (C57/B6-J $MPO^{-/-}$; MPO-KO) and wild-type (WT) mice were subcutaneously inoculated into the flank with mouse cancer cell lines. Anti-mouse-CD8 antibody was administered to some tumor-bearing mice in order to deplete $CD8^+$ T cells. Tumor growth was monitored. Mice were sacrificed, tumors were collected, weighed and measured. Single-cell suspensions were obtained from the tumors and flow-cytometry staining of immune populations was performed. *In vitro*: A549 human lung adeno-carcinoma cells were treated with MPO, and MPO subcellular localization, cancer cell proliferation and apoptosis were investigated. Peripheral blood mononuclear cells were isolated from human blood, and T cells were enriched using a commercial kit. T cells were treated with MPO, and T cell proliferation and activation were explored. Cellular localization of MPO in T cells was also investigated.
Results: So far, we have found convincing evidence for MPO acting in favor of tumor development. *In vivo*, MPO-KO mice displayed smaller tumors in comparison to the WT littermates. Remarkably, the analysis of the TME revealed an increased number of different lymphoid populations in the $MPO^{-/-}$ mice when compared to WT mice. $CD8^+$ T cell depletion reversed the reduction of the tumor size in the MPO-KO mice. *In vitro*, MPO was able to bind and internalize in A549 cells. Furthermore, A549 cells showed increased proliferation and reduced apoptosis after MPO treatment. Phosphorylation of Akt and Erk was increased in A549 cells stimulated with MPO. Finally, MPO was able to bind and internalize in human T cells, and interestingly, the proliferation of T cells was decreased after MPO treatment.

Discussion: MPO is the major protein in granules of neutrophils and is one of the key players in neutrophil function. The current project sheds light on the role of neutrophil-derived molecules as important players in the context of tumor growth and marks MPO as an enzyme within the TME that impacts NSCLC development. Further studies are needed to understand the functional significance of MPO localization in the cytoplasm and nucleus of cancer cells and T cells. Moreover, the use of specific MPO inhibitors *in vivo* is necessary in order to implement a therapeutic approach. Our findings suggest that MPO may play a role in the development of lung cancer either by regulating cancer cell function or by influencing immune-cell behavior.

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Keywords: myeloperoxidase – neutrophils – non-small-cell lung cancer

Author Index (Numbers refer to abstract no.)

Keyword Index (Numbers refer to abstract no.)

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