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Elena Pepeldjiyska

aus Sofia, Bulgarien

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Erstes Gutachten:	Prof. Dr. rer. nat. Dr. rer. biol. hum. habil. Helga Schmetzer, Dipl. Biol.
Zweites Gutachten:	Prof. Dr. Christian Ries
Drittes Gutachten:	Prof. Dr. Christoph Salat
Promovierter Mitbetreuer:	PD Dr. med. Klaus Metzeler
Dekan:	Prof. Dr. med. Thomas Gudermann
Tag der mündlichen Prüfung:	07.12.2023

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Eidesstattliche Versicherung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Thema

Role of immune regulatory mechanisms in AML and ALL

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München, den 01.06.2023 Elena Pepeldjiyska

Abbreviations

AML	acute myeloid leukemia
ALL	acute lymphoblastic leukemia
APC	antigen presenting cells
CAR	chimeric antigen receptor
CCR7	chemokine receptor 7
CD	Cluster of Differentiation
CD25	CD25 expression
$CD127^{low}$	low surface expression of the IL-7 receptor
CIK-cells	cytokine-induced killer cells
CLL	Chronic lymphocytic leukemia
CR	Complete Remission
CTX	Cytotoxicity Fluorolysis Assay
DC	Dendritic Cells
DC_{leu}	leukemia-derived Dendritic Cells
Deg	Degranulation Assay
DLI	Donor Lymphocyte Infusion
ECM	extracellular matrix
FasL	Fas ligand
FoxP3	Forkhead Box P3
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Good Manufacturing Practice
GVHD	Graft-vs-host disease
HSC	hematopoietic stem cell
HSCT	hematopoetic stem cell transplantation
ICS	Intracellular Cytokine Staining
IL-2	Interleukin-2
IL-7	Interleukin-7
IL-10	Interleukin-10

kDa	kilodalton
MHC	major histocompatibility complex
moDCs	monocyte ($CD14^+$) derived DCs
MLC	mixed lymphocyte culture
$MLC^{WB\text{-}DC(M)}$	mixed lymphocyte culture using WB after Kit-M treatment as “stimulator cells”.
$MLC^{WB\text{-}DC(Control)}$	mixed lymphocyte culture using WB without Kit-M treatment as “stimulator cells”.
MMPs	matrix metalloproteinases
MNC	mononuclear cells
NK-cells	Natural Killer cells
n.s.	not significant
PB	peripheral blood
PGE_1	Prostaglandin E_1
TGF-β	Transforming growth factor beta
TIMPs	tissue inhibitors of metalloproteinases
TNFα	Tumor-necrosis-factor alpha
TRAIL	TNF-related apoptosis inducing ligand
T_{reg}	regulatory T-cells
WB	whole blood

1. Publications

1.1 Publications Included in This Thesis

The following publications were summarized for this cumulative medical thesis in accordance with the examination rules of the medical faculty of the LMU Munich:

Publication I:

Leukemia derived dendritic cell (DC_{leu}) mediated immune response goes along with reduced (leukemia-specific) regulatory T-cells
Pepeldjiyska E, Lin L, Gao J, Seidel CL, Blasi C, Özkaya E, Schmohl J, Kraemer D, Schmid C, Rank A, Schmetzer H. Immunobiology. 2022 Jul;227(4):152237. doi: 10.1016/j.imbio.2022.152237. Epub 2022 Jun 11. PMID: 35749805. *IF 3,144*

Publication II:

Expression of surface-associated 82 kDa proMMP-9 in lymphatic leukemia blast cells differentially correlates with prognosis
Schmohl J, Moebius S, Guenther T, **Pepeldjyika E**, Seidel CL, Sutanto W, Schuster F, Kraemer D, Salih H, Hartmann A, Tischer J, Ries C, Schmetzer H. Anticancer Res. 41(8):3891-3898 doi:10.21873/anticanres.15184 (2021) *IF 2,48*

1.2 Other original Publications

Li L, Görgens A, Mussack V, **Pepeldjiyska E**, Hartz AS, Rank A, Schmohl J, Krämer D, Andaloussi SE, Pfaffl MW, Schmetzer H. *Description and optimization of a multiplex bead-based flow cytometry method (MBFCM) to characterize extracellular vesicles in serum samples from patients with hematological malignancies*. Cancer Gene Ther. 2022 Apr 27. doi: 10.1038/s41417-022-00466-1. Epub ahead of print. PMID: 35477770. *IF 5,98*

Rackl E, Li L, Klauer LK, Ugur S, **Pepeldjiyska E**, Seidel CL, Gunsilius C, Weinmann M, Doraneh-Gard F, Reiter N, Plett C, Amberger DC, Bojko P, Kraemer D, Schmohl J, Rank A, Schmid C, Schmetzer HM. *Dendritic Cell-Triggered Immune Activation Goes along with Provision of (Leukemia-Specific) Integrin Beta 7-Expressing Immune Cells and Improved Antileukemic Processes.* Int J Mol Sci. 2022 Dec 27;24(1):463. doi: 10.3390/ijms24010463. PMID: 36613907; PMCID: PMC9820538. *IF 6,2*

Li L, Mussack V, Görgens A, Pepeldjiyska E, Hartz AS, Aslan H, Rackl E, Rank A, Schmohl J, El Andaloussi S, Pfaffl MW, Schmetzer H. *The potential role of serum extracellular vesicle derived small RNAs in AML research as non-invasive biomarker*. Nanoscale Adv. 2023 Feb 20;5(6):1691-1705. doi: 10.1039/d2na00959e. PMID: 36926576; PMCID: PMC10012871. *IF 5,11*

1.3 Manuscripts submitted/in preparation for publication

S. Ugur, L.K. Klauer, C. Blasi, F. Doraneh-Gard, C. Plett, C. Gunsilius, D.C. Amberger, M. Weinmann, O. Schutti, Z. Fischer, E. Özkaya, M. Atzler, **E. Pepeldjiyska**, A. Völker, J. Schmohl, A. Rank5, C. Schmid, H.M. Schmetzer: *'Kit'-mediated blastmodulation to*

leukemia-derived DC significantly improves antileukemic activities in whole blood independent of AML-patients' subtypes. (in prep)

S. Bohlscheid, **E Pepeldjiyska**, L Li, J Gao, C Seidel, C. Blasi, E Özkaya, J Schmohl, D Kraemer, C Schmid, A Rank, HM Schmetzer *Kit-treatment of Antileukaemic T-cell responses can be predicted by compositions of regulatory T-cell subpopulations-under hypoxic and normoxic conditions* (in prep)

1.4 Contributions to conferences (Posters)

S. Ugur, L.K. Klauer, C. Blasi, F. Doraneh-Gard, C. Plett, C. Gunsilius, D.C. Amberger, M. Weinmann, O. Schutti, Z. Fischer, E. Özkaya, M. Atzler, **E. Pepeldjiyska**, A. Völker, J. Schmohl, A. Rank[5], C. Schmid, H.M. Schmetzer: *'Kit'-mediated blastmodulation to leukemia-derived DC significantly improves antileukemic activities in whole blood independent of AML-patients' subtypes.* EBMT, Bone Marrow Transplantation (2020)

L.Li, V.Mussack**, E.Pepeldjiyska**, A. S.Hartz, D.Krämer, A.Rank, C.Schmid E. Özkaya, S. Ugur, M.W.Pfaffl, H.M.Schmetzer: *Role of Exosomes as promotors or biomarkers to study activation of leukemia-derived dendritic cells (DCleu)-mediated antileukemic activation of adaptive and innate immune-reactive cells against AML-blasts.* EBMT, Bone Marrow Transplantation, A392 (2020)

L Li, V Mussack, **E Pepeldjiyska**, A Hartz, A Rank, C Schmid, E Özkaya, S Ugur, M Pfaffl, H Schmetzer P01.11 *Role of exosomes as promotors or biomarkers to study activation of leukemia-derived dendritic cells (DCleu)-mediated antileukemic activation of adaptive and innate immune-reactive cells against AML-blasts*. Journal for ImmunoTherapy of Cancer Oct 2020, 8 (Suppl 2) A13-A14; DOI: 10.1136/jitc-2020-ITOC7.24 (2020)

Co-Authors'confirmations

All co-authors signed a confirmation document, that Elena Pepeldjiyska has the permission to use the publications for her medical thesis. The Documents were submitted separately with this thesis.

3. Introduction

3.1 Leukemia

Acute leukemia is a clonal malignancy of the hematopoietic system that is characterized by loss of cell differentiation and uncontrolled proliferation of myeloid/lymphoid progenitor cells (Medinger & Passweg, 2017). To achieve a complete remission (CR) treatment involves intensive chemotherapy with or without hematopoietic stem cell transplantation (HSCT) followed sometimes by donor lymphocyte infusions (DLI). Nevertheless, the treatment remains challenging; in particular; due to high morbidity and mortality rates, patients over 65 years of age, who make up the majority of those with acute myeloid leukemia (AML), are not able to receive HSCT as a treatment option. Even though induction therapy offers a 70-80% chance of a CR in patients under 60, the relapse or persistence rates remain high and the prognosis is poor, with a 5-year survival rate of around 40-45% (Döhner et al., 2017; Leotta et al., 2022). Acute lymphoblastic leukemia (ALL) occurs predominantly in children and elderly patients. Prognosis is determined by immunophenotype, cytogenetics and molecular markers which affect the therapeutic approaches (e.g.; chemotherapy with or without HSCT) (Aldoss et al., 2019). Childhood ALL is treated with HSCT only in case of high-risk features (e.g.; unfavourable cytogenetics) or in case of relapse (Davis & Wistinghausen, 2019; Truong et al., 2021). Over the last decade the cure rates and survival outcomes for paediatric ALL patients have improved considerably, with 5-year survival rate of 89%. However, the survival rates for adult patients with ALL remain low, ranging from 20%-49% (Brown et al., 2021). Researchers are working on new forms of immunotherapy, such as monoclonal antibodies and chimeric antigen receptor (CAR) T-cells, that have the potential to improve the therapeutic strategies and outcomes for ALL (Malard & Mohty, 2020). Chronic lymphocytic leukemia (CLL) is the most common chronic leukemia in elderly patients. Median patient age is over 70 years at first diagnosis (Stauder et al., 2017). CLL is a condition in which small, mature B cells proliferate and build up in the blood, bone marrow, and lymphoid tissues due to defects in apoptosis and a clonal proliferation of these cells (Chiorazzi, 2007; Delgado et al., 2020). Treatment is individualized and indicated only in patients with active or symptomatic disease or with advanced Binet stage (Hallek & Al-Sawaf, 2021).

3.2 New therapy options for AML patients- DC/DC_{leu} based therapy

Alternative treatment options for AML patients are needed. Above all, immunomodulatory methods have shown promising results by (re)-activating the innate and adaptive immune system against leukemia (Moeinafshar et al., 2021). Lately, various (immune or chemo)

therapies against leukemia have been explored (e.g.; checkpoint inhibitors, chimeric antigen receptor (CAR) T-cells therapy, lymphocyte-promoting cytokines) (as summarized in Ansprenger et al., 2020 and Moeinafshar et al., 2021). In this thesis, we focused on the use of dendritic cells (DC) as a treatment alternative for AML patients. DCs are antigen presenting cells (APCs) of the innate immune system, which internalize, process and present antigens via major histocompatibility complex (MHC) I and II in lymphoid organs to cells of the innate and adaptive immune system. Only mature DCs, expressing chemokine receptor 7 (CCR7), are able to migrate to lymph nodes, where they can trigger both the adaptive and innate immune response leading to acquisition of leukemia-specific activity (Amberger et al., 2019; Gardner et al., 2020; Grabrucker et al., 2010). DC-based vaccines use monocyte derived dendritic cells (moDC) loaded with leukemic antigens, which are prepared under Good Manufacturing Practice (GMP)-conditions (Vago & Gojo, 2020). Leukemia derived DC (DC_{leu}) can be either produced from blasts ex vivo for adoptive transfer or could alternatively be generated directly in vivo using "DC-generating Kits" (e.g., Kit-I (GM-CSF and OK-432) or Kit-M (GM-CSF and PGE_1) (Amberger et al., 2019; Amberger & Schmetzer, 2020; Schwepcke et al., 2022) (European and USA Patents: 15 801 987.7-1118 and 15/956343). DC/DC_{leu} are defined by their ability to express both dendritic cell antigens and patient's individual leukemia antigens simultaneosly. DC/DC_{leu} can be generated from leukemic whole blood (WB) ex vivo using Kits without inducing blast proliferation and independently of the patient's age, gender, cytogenetic risk profile or AML subtype, therefore it represents a promising topic for ongoing research (Amberger & Schmetzer, 2020; Plett et al., 2022; Schwepcke et al., 2022).

3.3 Innate and adaptive immune system

The innate immune system includes antigen presenting cells (APC) (e.g.; macrophages, DCs), cytokine-induced killer (CIK), invariant natural killer T-cells (iNKT) and natural killer (NK) cells, which serve as primary defenders against tumors and pathogens (Mortezaee & Majidpoor, 2022; Robertson et al., 2014). NK-cells ($CD56^+CD3^-$) regulate the adaptive system by physical interactions between T- and DC- producing cytokines and chemokines (Vivier et al., 2011). CIK-cells ($CD56^+CD3^+$) possess characteristics and functions of both NK-cells and T-cells (Boeck et al., 2017; Schlegel et al., 2019). T- and B- cells are components of the adaptive immune system that play a role in tumor immunity through antigen-specific reactions and the generation of long-lasting immunological memory after the recognition of the pathogen/tumor by effector-memory responses (Netea et al., 2020). The

immune system's responses are controlled through various mechanisms: regulatory cytokines (such as Interleukin (IL)-10), tolerogenic dendritic cells, exosomes, and regulatory T-cells (Amberger & Schmetzer, 2020; L. Li et al., 2022; Pitt et al., 2014).

3.4 Regulatory T-cells

Regulatory T-cells (T_{reg}) are defined by their high or low expression of CD25 and the expression of the intracellular transcription factor Forkhead Box P3 ($FoxP3^{+}$). $FoxP3^{+}$ regulates T_{reg} development and function (Grover et al., 2021; C. Li et al., 2020; Schick et al., 2013). Studies have shown a strong correlation between FoxP3+ expression and low surface expression of the IL-7 receptor ($CD127^{low}$) on T_{reg}. $CD127^{low}$ can be used as a marker for identifying T_{reg} populations (Pepeldjiyska et al., 2022; Schick et al., 2013). From a clinical point of view T_{reg} are regarded as ambiguously. They play a role in blocking excessive immune responses in autoimmune diseases, transplantations, or graft-vs-host disease (GVHD) (DiPaolo et al., 2007; Guo et al., 2021; Zhang et al., 2010). However, high frequencies of T_{reg} can reduce the immune system's ability to fight cancer by suppressing immune effector cells (Grover et al., 2021; Mougiakakos et al., 2010). T_{reg} implement four distinct mechanisms to create an immunosuppressive environment including production of inhibitory cytokines such as Transforming growth factor beta (TGF-β) and IL-10, inhibition of mature DC, reduction of Interleukin (IL)-2 mediated metabolism and perforin-granzyme pathway as a mechanism to suppress the function of immune cells by inducing cell death (Grossman et al., 2004; Grover et al., 2021; Vignali, 2008). In doing so, T_{reg} cells are capable of reducing the anti-leukemic/leukemia-specific activity of immune cells (Pepeldjiyska et al., 2022).

3.5 Antileukemic (leukemia-specific and cytotoxic) activity

Antileukemic (leukemia-specific activity and cytotoxic) activity requires a compound interplay between immune cells and leukemic blasts. Several methods have been developed to detect and analyze the functionality of effector cells in a setting of antigen specific stimulation (Klauer et al., 2022; Pepeldjiyska et al., 2022). Activation and proliferation after antigen stimulation of T-cells can be identified by coexpression of CD69, CD71 or CD154 (Boeck et al., 2017). Intracellular Cytokine Staining (ICS) can be used to detect the production of leukemia-specific cells by measuring IFNγ or TNFα in different immune reactive cells, as determined by immunophenotyping (Deng & Mosmann, 2015). Additionally, degranulation assay (Deg) can be used to detect the blast cytotoxic capacity of effector cells by measuring the presence of lysosome-associated membrane protein-1

(LAMP-1, CD107a) on the cell surface (Pepeldjiyska et al., 2022; Olivo Pimentel et al., 2020; Schulte et al., 2008). A method for the detection of the cytotoxicity of effector cells is based on the cell-mediated target cell killing and assessed by a non-radioactive cytotoxicity assay (CTX). Target cells (e.g., blasts) and effector cells (previously stimulated or unstimulated) are co-cultured and the number of viable cells over the culture period is analysed. In the CTX the number of viable blasts labelled with fluorochrome-labelled antibodies after the influence of effector cells can be measured by performing target-effector cell incubations for 3 or 24 hours (Grabrucker et al., 2010). Lysis of target cells can occur through either a granule-dependent or a granule-independent mechanism. The granule-dependent pathway is fast-acting and results in cell death through the secretion of perforin and granzymes. The granule-independent mechanism is slower-acting. The death ligands FasL (Fas ligand) and TRAIL (TNF-related apoptosis inducing ligand) expressed by effector cells bind the target cell receptors initiating the extrinsic, the intrinsic or the mitochondrial pathways of apoptosis depending on the cell type (Martínez-Lostao et al., 2015; Tuomela et al., 2022). The use of CTX in conjunction with the ICS and Deg assays offers an advanced and compound examination of the functionality of different immune reactive cells, notably their leukemia-specific activity and cytotoxicity (Pepeldjiyska et al., 2022).

3.6 Role of matrix metalloproteinases (MMPs)

The matrix metalloproteinases (MMPs) is a large family of structurally and functionally related proteolytic enzymes that control the composition of extracellular matrix (ECM) and play a part in hematopoietic stem cell (HSC) migration and function (Saleh et al., 2021). Among them, MMP-9 effectively degrades denatured collagens (gelatine) and collagen type IV, the major ECM components, permitting progression, invasion, and metastasis of tumor cells (Juric et al., 2018; Mondal et al., 2020). Under normal physiological conditions, most of MMP-9 is secreted as a 92 kDa pro-form and its proteolytic activity is controlled by the inhibiting activity of the natural tissue inhibitors of metalloproteinases (TIMPs) (Cabral-Pacheco et al., 2020; Saleh et al., 2021). Previously a unique non-secreted 82 kDa variant of proMMP-9 on AML cells has been described which is expressed on the surface of blast cells (Ries et al., 2007; Schmohl et al., 2016). This 82 kDa proMMP-9 differs structurally and functionally from the ‘regular’ 92 kDa proMMP-9, suggesting a special role for the 82 kDa proMMP-9 in surface-associated proteolysis. Once activated, the 82 kDa proMMP-9 is only poorly inhibited by TIMP-1 (Ries et al., 2007; Schmohl et al., 2021). Therefore, overexpression of this TIMP-1-insensitive MMP-9 variant on the surface of malignant cells

may increase pericellular proteolysis and in this manner encourage cancer progression *in vivo* (Ries et al., 2007; Schmohl et al., 2016). Previous studies showed that surface expression of 82 kDa proMMP-9 on blast cells from peripheral blood (PB) represents a new independent marker of prognosis in AML patients (Ries et al., 2007; Schmohl et al., 2016). The role of MMP-9 requires further investigation especially in patients with ALL and CLL since its deregulation could be a potential target for cancer prognosis and treatment.

3.7 Outline of this doctoral thesis

This project investigates the roles of (leukemia-specific) immune cells (focussing on T_{reg}) and the role of 82kDa proMMP-9 in the context of antileukemic immunity. The first study (Pepeldjiyska et al., 2022) deals with the analytical characterization of leukemia-specific cells, especially T_{reg} in healthy and AML WB samples induced or not induced by blast modulating Kit-M (GM-CSF and PGE_1) pretreated WB after MLC and the relevance for mediating antileukemic responses. In our second study (Schmohl et al., 2021) we speculated that 82 kDa proMMP-9, when expressed on leukemic blasts, plays a pleiotropic role by activating or inactivating cytokines/chemokines or by proteolytic remodelling of ECM leading to the growth and infiltration of malignant cells. Therefore, we explored the association of this variant on the surface of leukemic cells from ALL and CLL patients with clinical and prognostic parameters.

2. Publications Included in this Thesis and Contributions

2.1 Publication I, Pepeldjiyska et al. 2022

Title: *Leukemia derived dendritic cell (DC_{leu}) mediated immune response goes along with reduced (leukemia-specific) regulatory T-cells*

Authors: Elena Pepeldjiyska, Lin Li, Jincheng Gao, Corinna L. Seidel, Christian Blasi, Erdem Özkaya, Jörg Schmohl, Doris Kraemer, Christoph Schmid, Andreas Rank, Helga Maria Schmetzer

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DC and DC_{leu} were generated ex vivo from 16 AML patients' samples with blastmodulatory Kit-M containing GM-CSF and PGE_1. Afterwards, Kit-M treated DC and DC_{leu} containing WB was co-cultured with patients' thawn T-cells in MLC and immune cells' composition after culture was analysed by flow cytometry. We performed a cytotoxicity assay, an intracellular staining assay and a degranulation assay to evaluate antileukemic and leukemia-specific functionality. Our group had already demonstrated in the past that Kit-M treatment of WB generates (mature) DC and DC_{leu} from leukemic WB, thereby activates T-cells' proliferation and differentiation (especially central memory T-cells) and decreases frequencies of T_{regs} (CD3+, CD4+, CD8+ T_{reg}) compared to control. Moreover, in *Pepeldjiyska et al., 2022* we showed that Kit-M pretreated WB increases leukemia-specific activity of immune cells (specifically intracellular TNFα/IFNγ production and CD107a expression) while decreases the frequencies of leukemia-specific T_{regs} compared to control. Lastly, we represented that pretreatment with Kit-M leads to improved cytotoxicity compared to control, which has a positive correlation with the leukemia-specific activity of T-cells, but a negative one with the (leukemia-specific) T_{regs} regardless of patients' clinical or diagnostic parameters. Overall, our results suggest that Kit-M pretreatment may help to reinstate the immune system's ability to fight AML by reducing the immunosuppressive (leukemia-specific) T_{regs} (as demonstrated with functional assays ICS, Deg, CTX) (Pepeldjiyska et al., 2022).

Contribution: Elena Pepeldjiyska performed most of the experiments, was responsible for data acquisition, interpretation and analysis including all statistical work. She drafted the manuscript together with Prof. Schmetzer.

2.2 Publication II, Schmohl et al. 2021

Title: *Expression of surface-associated 82 kDa proMMP-9 in lymphatic leukemia blast cells differentially correlates with prognosis*

Authors: Joerg Schmohl, Sabine Moebius, Thomas Guenther, Elena Pepeldjiyska, Corinna L. Seidel, Wishnu Sutanto, Friedhelm Schuster, Doris Kraemer, Helmut Salih, Amely Hartmann, Johanna Tischer, Christian Ries and Helga Schmetzer

Journal: Anticancer Research. 41(8):3891-3898 doi:10.21873/anticanres.15184 (2021) *IF 2,48*

In this project our group investigated the association of a membrane-bound 82kDa proMMP-9 with ALL and CLL. The 82kDa proMMP-9 expression profiles of PB-cells from 18 ALL patients and 21 CLL patients in blast/lymphocyte-rich phases of the disease were analysed by flow cytometry and results correlated with clinical parameters (e.g., risk groups, response to therapy, sex, age, or extramedullary disease). In ALL, mature B-linear blasts expressed higher frequencies of 82kDA proMMP-9 compared to T-linear blasts. Moreover, higher frequencies of 82kDa proMMP-9 were observed in elderly patients and in patients with relapse. Cases with extramedullary disease in either kidney or skin didn't show a different expression level of proMMP-9 compared to other patients. In CLL the 82kDa proMMP-9 expression did not correlate with any of the clinical parameters. Our results demonstrated that higher frequencies of 82kDa proMMP-9 expression on blast cells correlated with a more unfavourable ALL-subtype. Therefore, the 82kDa proMMP-9 expression could be used as a prognostic marker for ALL (Schmohl et al., 2021).

Contribution: Elena Pepeldjiyska contributed to statistical work, was responsible for the graphical presentation and contributed to the drafting of the results' part.

5. Summary

We investigated cellular and molecular mechanisms which could contribute to a better understanding of the pathogenesis of leukemia and to the development of potential treatment strategies.

The blastmodulatory Kit-M, composed of clinically approved drugs (GM-CSF and PGE_1), generates DC and DC_{leu} from leukemic WB. DC and DC_{leu} containing WB leads to specifically stimulated anti-leukemic (T)-cells after T-cell enriched MLC, giving rise to improved anti-leukemic immune response as already shown (Amberger et al., 2019; Ugur et al., 2022). In our first study (Pepeldjiyska et al., 2022) we focussed on the effect of Kit-M treated, DC/DC_{leu} containing patients' WB (n=16) on the provision of (leukemia-specific) immunosuppressive regulatory T-cells after MLC. We used functional assays such as cytotoxicity, intracellular cytokine staining and degranulation assays to show that Kit-M pretreated WB increases leukemia-specific activity of immune cells while significantly decreases (leukemia-specific) T_{reg} frequencies, going along with improved blast lysis. Thereby our results pointed to a shift from "suppressor to effector cells" mediated by Kit-M pretreatment. Altogether our study gives insight into T_{reg} functions, with respect to inhibiting antileukemic effects and potential modifications of these inhibitory mechanisms.

Another important mechanism of regulating antileukemic immune responses is associated with the MMP-9 expression on leukemic blasts. MMP-9 remodels the extracellular matrix and processes regulatory proteins, which may promote tumour growth, metastasis, and chemotherapy resistance (Mondal et al., 2020; Saleh et al., 2021). Considering the impaired inhibition mediated by its natural inhibitor TIMP-1, the previously reported non-secreted 82kDa proMMP-9 might affect the function of leukemic cells. In our second study (Schmohl et al., 2021) we performed flow-cytometric analysis of 82kDa proMMP-9 expression on ALL blasts (n=18) and CLL lymphocytes (n=21) from PB and correlated data with clinical parameters. We demonstrated that higher frequencies of 82kDa proMMP-9 expression on blast cells may correlate with a more unfavourable ALL-subtype, while in CLL no correlations with any clinical parameters were observed. The study highlights the correlation between 82kDa proMMP-9 expression on blast cells and ALL prognosis, which may contribute to selection of risk associated treatments.

My doctoral thesis concludes that further studies are needed to fully understand the pathophysiology of leukemia and underlines the role of (leukemia-specific) T_{reg} as well as of

the 82kDa pro-MMP-9 expression as potential regulatory prognostic markers and therapeutic targets in leukemia.

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6. Zusammenfassung

Wir untersuchten zelluläre und molekulare Mechanismen, die zum besseren Verständnis der Pathogenese von Leukämien und zur Entwicklung neue Behandlungsstrategien beitragen könnten.

Das blastenmodulatorische Kit-M, bestehend aus klinisch zugelassenen Arzneimitteln (GM-CSF und PGE_1), erzeugt DC und DC_{leu} aus Blasten in WB. Derartige DC und DC_{leu} führen nach T-Zell-angereicherter MLC zu spezifisch stimulierten antileukämischen (T)-Zellen, was zu einer verbesserten antileukämischen Immunantwort führt, wie bereits gezeigt wurde (Amberger et al., 2019; Ugur et al., 2022). In unserer ersten Studie (Pepeldjiyska et al., 2022) konzentrierten wir uns auf die Wirkung von mit Kit-M behandeltem, DC/DC_{leu} haltigem WB (n=16) von Patienten auf die Bereitstellung von immunsuppressiven regulatorischen T-Zellen. Wir verwendeten funktionelle Assays wie Zytotoxizitäts-, intrazelluläre Zytokin und Degranulationsassays, um zu zeigen, dass mit Kit-M vorbehandeltes WB die leukämiespezifische Aktivität von Immunzellen erhöht, während die (leukämiespezifischen) T_{reg}-Frequenzen signifikant verringert wird, was mit einer verbesserten Blastenlyse einhergeht. Unsere Ergebnisse weisen auf eine durch die Kit-M-Vorbehandlung vermittelte Verschiebung von „Suppressor- zu Effektorzellen“ hin. Insgesamt gibt unsere Studie einen Einblick in die T_{reg}-Funktionen im Hinblick auf die Hemmung antileukämischer Wirkungen und mögliche Modifikationen dieser Hemmmechanismen.

Ein weiterer wichtiger Mechanismus zur Regulierung antileukämischer Immunantworten ist mit der MMP-9-Expression auf leukämischen Blasten assoziiert. MMP-9 baut die extrazelluläre Matrix um und verarbeitet regulatorische Proteine, die Tumorwachstum, Metastasierung und Chemotherapieresistenz fördern können (Mondal et al., 2020; Saleh et al., 2021). In Anbetracht der beeinträchtigten Hemmung durch seinen natürlichen Inhibitor TIMP-1, könnte das früher beschriebene nicht-sezernierte 82kDa proMMP-9 die Funktion von Leukämiezellen erheblich beeinträchtigen. In unserer zweiten Studie (Schmohl et al., 2021) führten wir eine durchflusszytometrische Analyse der 82 kDa-proMMP-9-Expression auf ALL-Blasten (n=18) und CLL-Lymphozyten (n=21) aus PB durch und korrelierten die Daten mit klinischen Parametern. Wir konnten zeigen, dass eine höhere Expression von 82kDa proMMP-9 auf Blastenzellen mit einem ungünstigeren ALL-Subtyp korrelieren kann, während bei CLL keine Korrelationen mit klinischen Parametern beobachtet wurde. Die Studie hebt die Korrelation zwischen der 82-kDa-proMMP-9-Expression auf Blasten und der

ALL-Prognose hervor, was zur Auswahl von risikoassoziierten Behandlungen beitragen könnte.

Meine Doktorarbeit kommt zu dem Schluss, dass weitere Studien nötig sind, um die Pathophysiologie von Leukämie vollständig zu verstehen, und unterstreicht die Rolle von (Leukämie-spezifischen) T_{reg} sowie eine 82kDa pro-MMP-9-Expression auf Blasten als potenzielle regulatorische prognostische Marker und therapeutische Ziele bei der Behandlung von Leukämien.

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