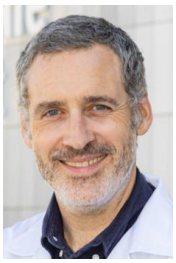
Michael Dengler (PI)/ Katharina Leithner (Co-PI)

Department of Internal Medicine, Division of Oncology and Otto Loewi Research Center (Pharmacology) and Medical University of Graz



Project Title:

Systemic Determinants of Lung Cancer Cell Fate and Therapy Response

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Lung cancer is the deadliest cancer worldwide and continues to pose a major clinical challenge due to rapid disease progression, early immune evasion, and limited durable responses to therapy (1-3). While tumor-intrinsic genetic alterations are key drivers of lung cancer, accumulating evidence indicates that systemic, age-associated changes in the hematopoietic system may critically influence lung cancer development and treatment outcome (4). One such change is clonal hematopoiesis of indeterminate potential (CHIP), a common age-related expansion of hematopoietic cell clones carrying somatic mutations, without clinical evidence of blood cancer.

Hypothesis and Objectives:

In this PhD project, the candidate will investigate how CHIP-driven alterations contribute to lung cancer development and therapeutic response. The central hypothesis is that CHIP-associated immune cell populations promote chronic inflammation that fuels tumor growth and/or reduces treatment efficacy. A particular focus will be on how CHIP-induced inflammatory signals modulate cellular stress responses and cell fate decisions, and how metabolic reprogramming contributes to these processes.

Methodology:

The PhD candidate will employ state-of-the-art lung cancer model systems, including genetically engineered and transplantation-based mouse models, combined with in vivo and ex vivo CRISPR-Cas9 genome engineering (*5, 6*). Advanced ex vivo co-culture systems and primary patient-derived lung cancer samples will be used to validate mechanistic findings in clinically relevant settings. Multiomics approaches, including transcriptomics and metabolomics, will be applied to define CHIP-induced alterations and to link them to deregulated cellular stress responses and cell death pathways.

A key translational goal of the project is the identification and preclinical evaluation of novel therapeutic strategies targeting CHIP-induced signaling pathways within the tumor. By integrating experimental findings with patient-derived data, the PhD candidate will contribute to the identification of new therapeutic vulnerabilities relevant to personalized lung cancer treatment. This project offers comprehensive training at the interface of cancer biology, immunology, and

metabolism, with direct exposure to patient material, advanced in vivo models, and cutting-edge genome engineering and multi-omics technologies.

References:

1. F. Bray *et al.*, Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **74**, 229-263 (2024).

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4. O. Pich *et al.*, Tumor-Infiltrating Clonal Hematopoiesis. *N Engl J Med* **392**, 1594-1608 (2025).

5. A. L. Meinhardt *et al.*, The BCL-2 family member BOK promotes KRAS-driven lung cancer progression in a p53-dependent manner. *Oncogene* **41**, 1376-1382 (2022).

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Alexander Deutsch (PI)/ Katharina Prochazka (Co-PI)

Department of Internal Medicine, Division of Hematology, Medical University of Graz

Project Title:

Dietary Regulation of NR4A1 as a Strategy to Boost CAR-T Cell Therapy Responses

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Chimeric antigen receptor (CAR) T cell therapy has emerged as a highly effective treatment for patients with aggressive B cell lymphomas who relapse or are refractory to standard therapies. However, in contrast to other B cell malignancies, response rates in aggressive lymphomas remain limited to approximately 50%, underscoring the need to identify modifiable factors that enhance therapeutic efficacy[1]. Preliminary data from our laboratory indicate that the nuclear receptor NR4A1 exerts tumor-suppressive functions by regulating immune-evasive processes in aggressive B cell lymphomas[2–4]. Dietary interventions have been shown to modulate NR4A1 expression[5] and may thereby influence CAR-T cell function and therapeutic response.

Hypothesis and Objectives:

Dietary restriction is hypothesized to enhance CAR-T cell therapy efficacy in aggressive B cell lymphoma by modulating NR4A1-dependent metabolic and immune pathways in both tumor cells and CAR-T cells. This project aims to define the role of NR4A1 in lymphoma–CAR-T cell interactions under diet-mimicking metabolic conditions, to assess how nutrient availability shapes CAR-T cell function ex vivo, and to determine whether dietary restriction improves therapeutic efficacy in vivo using isogenic NR4A1-proficient and NR4A1-deficient lymphoma mouse models. In parallel, samples from CAR-T–treated lymphoma patients will be analyzed to characterize NR4A1 expression and transcriptional signatures and to correlate these with metabolic parameters, nutritional status, and clinical response, thereby providing a translational framework for rationally combining dietary interventions with CAR-T cell therapy.

Methodology:

The PhD student will primarily focus on preclinical experimental models, integrating ex vivo and in vivo approaches to dissect NR4A1-dependent metabolic regulation of CAR-T cell therapy. A murine CAR-T cell production platform will be established to generate antigen-specific CAR-T cells for use in syngeneic, isogenic NR4A1-proficient and NR4A1-deficient lymphoma mouse models subjected to controlled dietary regimens, followed by CAR-T cell therapy and comprehensive assessment of tumor

growth, survival, CAR-T cell persistence, immune phenotypes, and metabolic alterations. In parallel, ex vivo metabolic conditioning studies will be performed using defined media formulations mimicking distinct dietary states to assess CAR-T cell and lymphoma cell function, including activation, proliferation, cytotoxicity, exhaustion, metabolic profiling, and NR4A1 activity. Patient-derived samples from a prospective CAR-T–treated lymphoma cohort will additionally be incorporated into ex vivo assays to enable translational analyses and to directly relate NR4A1 expression and metabolic states to CAR-T cell function and clinical response, thereby informing the rational design of combined dietary restriction and CAR-T cell therapy protocols.

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1) Neelapu SS, Jacobson CA, Ghobadi A, et al. Five-year follow-up of ZUMA-1 supports the curative potential of axicabtagene ciloleucel in refractory large B-cell lymphoma. Blood. 2023 May 11;141(19):2307-2315. doi: 10.1182/blood.2022018893.
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Sayantanee Dutta (PI)/ Andreas Reinisch (Co-PI)

Department of Internal Medicine, Division of Hematology and Department of Blood Group Serology and Transfusion Medicine Medical University of Graz

Project Title:

Elucidating the role of intrinsic Inflammation in Myeloid Leukemogenesis

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Inflammation promotes multiple hallmarks of cancer through both extrinsic and intrinsic mechanisms, including the induction of growth and angiogenic factors, sustained proliferative signaling, resistance to cell death, and suppression of antitumor immunity. In hematologic malignancies, chronic inflammation has been implicated in the selection of pre-malignant clones and in the progression of myelodysplastic syndromes (MDS) to acute myeloid leukemia (AML). As a central mediator of inflammatory responses, tumor necrosis factor receptor (TNFR) signaling plays a critical role in the regulation of normal hematopoiesis. Our previous work demonstrated that activation of intrinsic inflammatory signaling via TNFR1 selectively eradicates leukemic stem cells and induces deep, durable disease remission in vivo. Within the framework of the doctoral program TIMO (Translational Immunology and Metabolism in Oncology), this project aims to dissect the role of TNFR-mediated intrinsic inflammation in pre-leukemic and leukemic cells. Using complementary molecular, in vitro, and in vivo approaches, the candidate will elucidate how pro-inflammatory signaling through TNF receptors shapes the trajectory of myeloid leukemogenesis.

Hypothesis and Objectives:

Our previous work showed that activating pro-inflammatory TNFR signaling in primary AML blasts triggers both cell death and differentiation of leukemic stem cells (LSC). Notably, the same stimulus promotes proliferation of healthy hematopoietic stem and progenitor cells (HSPCs), highlighting a therapeutic window in which leukemic cells can be targeted while preserving normal hematopoiesis.
The PhD project aims to:

1. Identify actionable molecular pathways that cooperate with tumor-suppressive TNFR signaling to mediate antileukemic effects
2. Dissect the tumor-suppressive mechanisms of TNFR signaling in leukemia progression.
3. Elucidate how pro-inflammatory signals via TNF receptors impact healthy HSPCs and leukemic hematopoiesis.

This project lies at the interface of molecular oncology and precision medicine, aiming to uncover mechanistic insights that can inform the development of patient-tailored therapies for high-risk AML.

Methodology:

The project will leverage a combination of molecular, cellular, and in vivo approaches to address the proposed questions.

Key experimental systems and techniques include:

- **Genetic engineering using CRISPR-Cas9** in a panel of AML cell lines to investigate TNFR superfamily members and their downstream signaling complexes.
- **Genome wide CRISPR screen:** to identify pathways cooperating with TNFR1 signaling
- **Ex vivo culture models** using both healthy human HSPCs and patient-derived AML progenitor cells to study impact of intrinsic inflammatory signaling in a controlled setting.
- **In vivo models**:
    - Syngeneic murine bone marrow transplantation using gene-targeted mouse strains.
    - **Patient-derived xenograft (PDX) models** to evaluate the role of TNFR signaling in AML cell death and differentiation in a human-relevant context.

References:

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Philipp Jost (PI)/ Katarina Vizar Cisarova (Co-PI)

Department of Internal Medicine, Division of Oncology, Medical University of Graz

Project Title:

Immunologic and metabolic consequences of highly recurrent chromosomal 1q21 amplifications in lung cancer patients

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer-related deaths worldwide (1). Genetic analysis and novel treatment concept both heavily focus on gain-of-function point mutations in driver oncogenes. Yet, our recent work has identified alternative but highly recurrent genomic aberrations, namely chromosomal gains of chromosome 1q21, resulting in the overexpression of >300 genes in lung adenocarcinoma patients (2). Since several key genes involved in metabolic processes (*MCL1, LAMTOR2, CRTC2*) and immune cell activation (*IL6R, PSMD4*) are located in the 1q21 amplified region, it is conceivable that evolutionary trajectories select for cancer cells harboring 1q21 gains to metabolically and immunologically reprogram the tumor and/or its microenvironment.

Hypothesis and Objectives:

We hypothesize that clonal selection for 1q21 is linked to increased metabolic pressure following p53 loss, leading to a modified tumor immune microenvironment driven by p53-mediated proinflammatory cytokine signaling (3). The primary aim of this proposal is to characterize and dissect the 1q21 region in lung cancer, and subsequently pinpoint potential candidate genes within the 1q21 gains associated with immunologic signaling and metabolic reprogramming. Our final ambition is to identify novel potentially actionable (druggable) targets based on genomic alterations on 1q21 in lung cancer.

Methodology:

The project is encompassing *in silico*, *in vitro* and *in vivo* components. The foundation for each category has been well established; with *in silico* pipelines and advanced CRISPR-Cas9-based techniques forming the basis (2,4). The PhD student will analyze published next generation sequencing datasets of lung cancer patients to identify and characterize the commonly amplified region of 1q21 as well as to evaluate the association of candidate genes located on gains of 1q21 with immunologic signaling and metabolic reprogramming and to determine patterns of tumor immune cell infiltration. Identified candidate mechanisms will be functionally evaluated *in vitro* and *in vivo* using cell lines via

pharmacological inhibition or shRNA-based repression as well as our fully established CRISPR-Cas9 lung cancer mouse model.

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1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68(6):394–424.

2. Munkhbaatar E, Dietzen M, Agrawal D, Anton M, Jesinghaus M, Boxberg M, et al. MCL-1 gains occur with high frequency in lung adenocarcinoma and can be targeted therapeutically. Nat Commun. 2020 Sep 10;11(1):4527.

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4. Agrawal D, Cisarova K, Vosberg S, Allmendinger F, Munkhbaatar E, Dandachi N, et al. Aberrant methylation limits antitumoral inflammation in lung adenocarcinoma by restricting RIPK3 expression. Sci Adv. 2026 Jan 23;12(4): eadz9227.

Julia Kargl (PI)/ Gudrun Absenger (Co-PI)

Otto Loewi Research Center (Pharmacology) and Department of Internal Medicine, Division of Oncology, Medical University of Graz

Project Title:

Metabolic adaptation of neutrophil subsets in LKB1-deficient lung cancer

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical co-supervision (**link**).*

Background: Loss of the tumor suppressor LKB1 is a frequent genetic alteration in non-small cell lung cancer (NSCLC). These tumors show major metabolic changes, including increased glycolysis and lactate accumulation in the tumor microenvironment (TME). At the same time, LKB1-deficient tumors are poorly infiltrated by cytotoxic T cells and enriched for immunosuppressive cells such as neutrophils, contributing to immune evasion and resistance to immune checkpoint therapy (1). Our preliminary data indicate that neutrophil subsets display metabolic plasticity, including altered mitochondrial function and prolonged survival, which may directly support tumor progression.

Hypothesis and Objectives: We hypothesize that neutrophil subsets exhibit distinct metabolic states, and that phenotypic and functional changes in neutrophils result from their adaptation to the local environment. This study aims to unravel the interplay between LKB1 loss, the metabolic and proteomic consequences in the TME, and its role in shaping neutrophil phenotypes in lung cancer patients.

Methodology: To associate metabolic rewiring with neutrophil recruitment and function, the PhD student will combine the analyses of in-house and public NSCLC datasets with experimental work on patient material and in vivo models (2, 3). Neutrophil metabolism will be studied using state-of-the-art approaches, including SCENITH, extracellular flux analysis (Seahorse), and mass spectrometry. Pharmacological inhibition of mitochondrial respiration, glycolysis, lipid and amino-acid metabolism will be applied to test how metabolic pathways control neutrophil-mediated immunosuppression. Functional validation, including immunosuppression, activation, degranulation, NETosis and ROS production will be performed using primary neutrophils, CRISPR-Cas9-edited HSPC-derived neutrophils and patient-derived tumor models.

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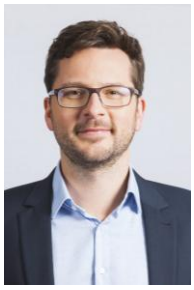
1 Koyama S et al., (2016) *Cancer Research*; doi: 10.1158/0008-5472.CAN-15-1439.

2 Kargl J et al., (2017) *Nature Communications*; doi: 10.1038/ncomms14381.

3 Kargl J et al., (2019) *JCI Insight*; doi: 10.1172/jci.insight.130850.

Jelena Krstic (PI)/ Gabriel Rinnerthaler (Co-PI)

Gottfried Schatz Research Center (Cell Biology, Histology and Embryology) and Department of Internal Medicine, Division of Oncology, Medical University of Graz



Project Title:

Impact of chemotherapy-induced metabolic rewiring on immunotherapy response in triple negative breast cancer

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Recently approved neoadjuvant therapy combining the anti-PD1 immune check point inhibitor (ICI) pembrolizumab with an anthracycline-containing polychemotherapy regimen presents a major step forward in the treatment of triple negative breast cancer (TNBC) [1]. As PD-L1 status alone is insufficient as a predictive biomarker of patient response, there is an unmet need to better understand the tumor microenvironment (TME) and improve identification of responders. Due to intrinsic heterogeneity, chemotherapy (CT) can inflict distinct metabolic reprogramming in the TME of different patients with TNBC. Besides that, conventional CT agents are also known to harbor immunogenic properties. For instance, anthracyclines can trigger an immunogenic cell death that engages the adaptive immune system. How metabolic adaptation to anthracyclines impacts patient response to adjunct ICI therapy is still unknown [2,3].

Hypothesis and Objectives:

We hypothesize that anthracyclines inflict differential metabolic rewiring of TNBC cells originating from different patients, subsequently affecting the TME and overall response to ICIs. Our goal is to predict the patient-specific response to CT/ICI combination therapy based on the metabolic changes in the TME. We will investigate the metabolic rewiring of the patient-specific TNBC upon anthracycline treatment, and how this affects the response to pembrolizumab.

Methodology:

The PhD student will establish organoid lines from biopsies obtained from patients with TNBC. The organoids will then be treated with an anthracycline (e.g., epirubicin) and their metabolic rewiring will be assessed by energetic profiling using Seahorse™ analyzer and by RNA sequencing analyzing metabolism-relevant gene expression. To study the responsiveness to pembrolizumab, we will perform co-culture experiments utilizing organoids and patient-matched immune cells, and conditioned media from control and CT-treated organoids will be used in T-cell migration and activation assays. Moreover, the PhD student will correlate the expression of metabolism-relevant

genes in TNBC biopsies with immune infiltration and response to combination therapy using multiplex imaging techniques and single cell RNAseq. In addition, the candidate will have a hands-on opportunity to learn how clinical trials investigating polychemotherapy regimens in patients with breast cancer are designed and performed through the ongoing clinical studies led by co-supervisor Dr. Rinnerthaler [4]. To investigate how anthracyclines modify the metabolite and cellular composition of the TME *in vivo*, the student will make use of a syngeneic mouse model (orthotopic transplantation of 4T1 mouse TNBC cell line). Blood plasma and cancer interstitial fluid metabolome will be analyzed via LC-MS, and single cell RNAseq will be used to assess the cellular composition of the TME [5,6].

References:

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Katharina Leithner (PI)/ Michael Dengler (Co-PI)

Otto Loewi Research Center (Pharmacology) and Department of Internal Medicine, Division of Oncology, Medical University of Graz

Project Title:

Metabolic alterations in lung cancer cells and mechanisms of adaptation to the tumor microenvironment

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Cancer cells rewire their metabolism to support tumor growth[1]. Lung cancer is an aggressive cancer with a mostly glycolytic phenotype. Still, lung cancer cells are able to adapt to glucose deprivation, e. g. by utilizing initial steps of gluconeogenesis, as shown by our group[2]. The mechanisms of adaptation of lung cancer cells to their nutrient- and oxygen deprived, high lactic acid microenvironment are still poorly understood.

Hypothesis and Objectives:

To clarify, which vulnerabilities emerge in microenvironmental stress conditions, we plan to investigate adaptations in lung cancer cells under low glucose and/or high lactate conditions and in different oxygen environments. Moreover, the role of the adaptive pathways on tumor cell interactions with immun cells will be investigated.

Methodology:

Stable-isotope resolved metabolomics using ^{13}C-glucose and other tracers will be performed using gas chromatography-mass spectrometry (established in the lab) or liquid chromatography-mass spectrometry (in collaboration with Thomas Eichmann, Core Facility Mass Spectrometry) in cancer cell lines and short-term explants from freshly removed non-small cell lung cancer. CRISPR-Cas9 mediated knockout will be used to analyze the impact of de-regulated pathways on cell metabolism, proliferation and cell death. The *in vivo* relevance of de-regulated pathways will be addressed using cancer cell xenografts in mice. Moreover, we will assess, whether blockade of the adaptive pathways in the cancer cells alters activities of co-cultured human macrophages or anti-tumor immunity in T-cell activation assays.

References:

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Katharina Prochazka (PI)/ Alexander Deutsch (Co-PI)

Department of Internal Medicine, Division of Hematology, Medical University of Graz

Project Title:

Nutritional and Metabolic Biomarkers Predicting CAR-T Cell Therapy Response in Aggressive B-Cell Lymphoma

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Chimeric antigen receptor (CAR) T cells therapy has shown significant clinical efficacy in aggressive lymphoma patients, who relapsed or experience a refractory (r/r) course under standard therapy. Compared to other B cell malignancies, response rates to this immunotherapy are around 50% in aggressive lymphoma1. In addition to variable efficacy, CAR T-cell therapy in diffuse large B-cell lymphoma (DLBCL) is associated with a distinct and clinically relevant toxicity profile, predominantly characterized by cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS), as well as prolonged cytopenias, infectious complications, and long-term B-cell aplasia with subsequent hypogammaglobulinemia. The incidence, severity, and temporal pattern of these toxicities vary considerably between CAR T-cell products and are further modulated by baseline disease characteristics and host-related factors2-4, underscoring the need for improved patient stratification and predictive biomarkers.

Hypothesis and Objectives:

We would like to investigate, how baseline and early on-treatment nutritional, metabolic, and microbiome-derived biomarkers, integrated with lymphoma specific NR4A1 expression/status, can predict CAR-T therapy response and toxicity in aggressive B-cell lymphoma. Consecutively, our aim is to define a “metabolic readiness score” that is modifiable by dietary interventions.

Methodology:

In a prospective analysis, the PhD-student will perform a longitudinal collection of various laboratory and metabolic parameters with a focus on microbiome-analysis. This will include DNA isolation, PCR and bioinformatics analysis and interpretation. She/he will integrate the results in a bioinformative model to predict outcome of CAR-T-cell patients dependent on their metabolic fitness.

References:

1) Neelapu SS, Jacobson CA, Ghobadi A, et al. Five-year follow-up of ZUMA-1 supports the curative potential of axicabtagene ciloleucel in refractory large B-cell lymphoma. Blood. 2023 May 11;141(19):2307-2315. doi: 10.1182/blood.2022018893.
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Andreas Prokesch (PI) / Martin Wagner (Co-PI)

Gottfried Schatz Center for Cell Signaling, Metabolism and Aging and
Clinical Department of Gastroenterology und Hepatology
Medical University of Graz

Project Title:

Mechanisms and translation of p53-mediated synergy between glucose-limiting diets and checkpoint therapy in hepatocellular carcinoma (HCC)

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

While an immune checkpoint combination (atezolizumab/bevacizumab) recently advanced HCC therapy, more than two thirds of HCC patients do not respond to this treatment [1], demanding novel combinatorial approaches. Low carbohydrate, ketogenic diets (LC-KDs) are increasingly established as adjuvant cancer therapy [2,3]. Our previous preclinical study showed that systemic HCC therapy response can be improved by combination with glucose restriction through fasting regimens in a p53-dependent manner [4]. Moreover, both p53, through regulating checkpoint protein expression [5], and LC-KDs, through enhancing T cell-mediated immunosurveillance [6,7], were recently implicated in improved checkpoint therapy outcomes in various cancers.

Hypothesis and Objectives:

We hypothesize that LC-KDs improve the efficacy of checkpoint therapy by rewiring the tumor metabolic and immune landscape of HCC. We will further test the impact of p53 hotspot mutations on the synergism of LC-KDs and checkpoint therapy in relevant immune-competent mouse and patient-derived models.

Methodology:

The PhD student will utilize our established and validated mouse HCC cell lines (Hepa1-6, Hep53.4) harboring the clinically relevant p53 hotspot mutations R175H, R249S, and R273H (CRISPR knock-in). These will be used for syngeneic, orthotopic implantations followed by checkpoint treatment alone or in combination with fasting-mimicking diet (FMD [4]) or ketogenic diet (KD) [3]. Tumor growth will be determined, and tumor samples will be subjected to single cell RNA-seq, immunofluorescence, and bulk metabolomics to interrogate the immune/metabolic landscape with a special focus on tumor associated macrophages [8]. p53 ChIP-seq will be done to define the targetomes of the hotspot mutations upon treatment, which will be confirmed in patient samples [9]. The student will further work with patient-derived HCC organoids and precision-cut liver slices (PCLSs [10]) from biopsies obtained through the clinical co-supervisor. HCC organoids, co-cultured with patient-matched immune cells (e.g. CD8+ T cells [11]), and PCLCs will be treated with checkpoint inhibitors in

combination with the ketone metabolite ß-hydroxybutyrate, with different glucose restrictions. Tumor-immune cell interactions depending on treatment combination will be evaluated with multicolor immunofluorescence and metabolite fluxes. Sequencing of the p53 locus in patient-derived models will relate to results in mice. Based on the results obtained, the student will then participate in design of an investigator-initiated clinical trial to test novel combination therapy approaches in HCC patients. Hence, this study will advance precision oncology by establishing modalities and mechanisms of the combination of glucose-limiting, ketogenic interventions with checkpoint therapy in HCC in dependence of patients' p53 status.

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Andreas Reinisch (PI)/ Sayantanee Dutta (Co-PI)

Department of Internal Medicine, Division of Hematology and Department of Blood Group Serology and Transfusion Medicine Medical University of Graz

Project Title:

Dissecting the Role of Inflammation in TET2 mutant Clonal Hematopoiesis Fitness

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Clonal hematopoiesis (CH) is characterized by the age-related expansion of hematopoietic stem and progenitor cell (HSPC) clones harboring somatic mutations, most frequently in the epigenetic regulator TET2.[1] While these mutations are found in the blood of healthy aging individuals, they significantly increase the risk for hematologic malignancies and inflammatory comorbidities, such as cardiovascular disease.[2,3]

Recent evidence suggests that the expansion of these clones is not merely stochastic but is driven by a shift in the fitness landscape of the bone marrow. Chronic inflammation - often associated with aging - serves as a decisive selective pressure. While an inflammatory milieu typically induces exhaustion or apoptosis in wild-type (WT) HSPCs, TET2-mutant cells exhibit an "inflammatory resilience," allowing them to outcompete healthy cells.[4,5] This project aims to identify the genetic dependencies that allow TET2-mutant cells to survive and proliferate under these hostile conditions, using unbiased screening and competitive models.

Hypothesis and Objectives:

TET2-mutant HSPCs possess a distinct genetic program that confers a cell-intrinsic resistance to inflammatory-induced exhaustion, providing a competitive fitness advantage that can be neutralized by targeting specific survival pathways.

The PhD project aims to:

1. **Quantify the selective advantage of *TET2*-mutant human HSPCs in vitro and in vivo.** You will determine how different inflammatory cytokines alter the selection of mutant cells in head-to-head competition with wild-type counterparts.
2. **Identify molecular drivers of inflammatory resistance.** You will perform scRNAseq analysis and functional CRISPR screen in human *TET2*-mutant cells to pinpoint the specific genes required for clonal persistence under chronic inflammatory stress.

3. **Exploid potential therapeutic targets to halt clonal expansion.** You will test whether the genetic dependencies identified in Aim 2 can be exploited to halt clonal expansion under conditions of systemic inflammation.

Methodology:

The project will leverage a combination of **molecular, cellular, and in vivo approaches** to address the proposed questions. Key experimental systems and techniques include:

- **Isolation and ex vivo culture** of primary human CD34+ HSPCs
- **Cellular Engineering** of primary human CD34+ HSPCs using CRISPR/Cas9-based technologies including Base Editing
- **In vitro competition and fitness modeling**
- **Humanized hematopoietic xenotransplantation assays**
- **scRNAseq**

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Beate Rinner (PI)/ Joanna Szkandera (Co-PI)

Diagnostic & Research Center of Pathology and Department of Internal Medicine, Division of Oncology, Medical University of Graz

Project Title:

Epigenetic and metabolomic alterations in translocation-related sarcomas

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Sarcomas are a heterogeneous, rare, and complex group of neoplasms of mesenchymal origin with frequent metastasis. They present in various age groups, often in adolescents and young adults, with wide variations in anatomic sites, subtypes, and prognosis. Translocation-related sarcomas (TRSs) are distinct subtypes driven by specific gene fusions[1]. Metastases determine patient outcomes and their treatment is particularly challenging. Many types of sarcoma have come to be considered as predominantly epigenetic diseases, rendering the involved mechanisms promising targets for innovative anticancer strategies [2-3]. The tumor microenvironment (TME) plays a crucial role in sarcoma progression, influencing tumor growth, immune evasion, and therapy resistance through complex interactions between cancer cells and stromal components such as cancer-associated fibroblasts (CAFs)[4]. Here, we plan to address this question by using and extending our recently established patient-derived TRS models with primary, metastatic, corresponding immortalized cancer-associated fibroblasts, and skin fibroblast cell lines from the same patient[5].

Hypothesis and Objectives:

We hypothesize that metastatic TRS cells undergo distinct epigenetic and metabolic alterations that drive aggressive tumor behavior and may serve as potential molecular targets for novel treatment strategies. Given the crucial role of the TME in tumor progression, immune evasion, and therapy resistance, we aim to comprehensively characterize epigenetic and metabolic differences between patient-derived primary and metastatic TRS tissues, as well as their corresponding cell lines, including CAFs and other TME components. By integrating these analyses, we seek to unravel key regulatory mechanisms that contribute to TRS progression and metastasis, ultimately identifying novel therapeutic vulnerabilities.

Methodology:

Preliminary data indicate pronounced epigenetic differences between metastatic and primary translocation-related sarcoma (TRS) cell lines, accompanied by a markedly increased glycolytic rate in metastatic models. Building on these findings, the PhD project aims to investigate the interplay between epigenetic regulation and metabolic reprogramming during sarcoma progression using

established patient-derived TRS cell models. The PhD student will employ pharmacological modulation of epigenetic regulators, focusing on methylating agents targeting protein arginine methyltransferases (PRMTs), in combination with genetic silencing or pharmacological inhibition of deregulated metabolic pathways. The effects on cell viability, proliferation, and induction of cell death will be assessed using standard in vitro assays, including flow cytometry–based analyses, apoptosis and cell cycle assays, and metabolic activity measurements. Changes in the metabolome as well as DNA and arginine methylation patterns will be analyzed using molecular and biochemical approaches. To validate and mechanistically dissect metabolic alterations, stable isotope tracing experiments will be performed in cell lines cultured with uniformly 13C-labeled glucose or glutamine, enabling detailed analysis of central carbon and amino acid metabolism. Targeted and untargeted metabolomic analyses will be complemented by gene and protein expression studies of candidate regulators involved in epigenetic and metabolic crosstalk, using quantitative PCR, Western blotting, and immunodetection-based methods. In addition, the PhD candidate will establish and characterize further sarcoma models to determine whether metastatic lesions consistently differ from primary tumors with respect to DNA methylation, arginine methylation, and metabolic profiles. The most promising TRS model will subsequently be evaluated in vivo using orthotopic xenograft models combined with advanced preclinical imaging technologies.

Overall, the project provides comprehensive training ranging from basic and advanced cell culture techniques to molecular, metabolic, and epigenetic analyses, as well as translational in vivo models, offering the PhD candidate broad methodological expertise and insight into tumor biology across experimental scales.

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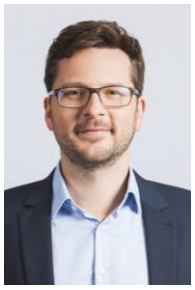


Gabriel Rinnerthaler (PI)/ Katarina Vizar Cisarova (Co-PI)

Department of Internal Medicine, Division of Oncology, Medical University of Graz

Project Title:

Impact of androgen and estrogen receptor signaling on immunological and metabolic pathways in breast cancer

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Estrogen receptor–positive (ER+) breast cancers, commonly referred to as luminal breast cancers, represent the most frequent molecular subtype of breast cancer[1]. In addition to dominant estrogen receptor signaling, a substantial proportion of luminal tumors also co-express the androgen receptor (AR). While ER signaling has been extensively studied and is the cornerstone of endocrine therapy, the biological and clinical relevance of concurrent AR and ER signaling, particularly with regard to tumor immunology and metabolism, remains poorly understood[2].

Aromatase inhibitors (AIs) constitute a key component of treatment for both early and metastatic ER+ breast cancer[3,4]. They reduce estrogen by blocking the conversion of androgens into estrogen in adipose tissue. As a result, estrogen decreases and the levels of androgen increase, potentially leading to increased activity of AR in cancer cells[5,6].

ER+ breast cancer tumors are typically considered immunologically “cold” with weak immune reposnses, which explains the limited success of immunotherapy in these patients[7]. It is currently unclear whether the hormonal changes caused by AIs cause favorable or unfavorable effects on tumor immunological and metabolic programs, and how this may influence antitumor immunity and clinical outcomes.

Hypothesis and Objectives:

Concurrent AR and ER signaling modulates immunological and metabolic pathways in luminal breast cancer, thereby shaping the tumor immune microenvironment and influencing recurrence-free survival. Aromatase inhibitor therapy induces measurable immunometabolic reprogramming through altered steroid hormone signaling.

Objectives

1. To characterize the interaction between AR and ER signaling and immunological and metabolic pathways in ER+ breast cancer.
2. To evaluate the prognostic relevance of AR- and ER-associated immunometabolic signatures with respect to recurrence-free survival.
3. To assess therapy-induced immunometabolic changes following short-term aromatase

inhibitor treatment using paired tumor samples.

4. To functionally validate key AR/ER-driven immunometabolic pathways in experimental model systems.

Methodology:

This project integrates translational-clinical, bioinformatic, and experimental approaches, supported by complementary expertise of the main PI and the co-PIs.

The PhD student will perform comprehensive gene expression analyses using publicly available datasets (e.g., TCGA, METABRIC) as well as tumor samples derived from clinical trials. Expression patterns of androgen and estrogen receptor–associated gene programs will be correlated with immunological signatures (e.g., immune cell infiltration, immune checkpoint expression) and metabolic pathways. These molecular features will be linked to clinical endpoints, with a particular focus on recurrence-free survival. A key component of the project will be the analysis of paired tumor samples obtained from treatment-naïve patients and from the same patients after short-term neoadjuvant aromatase inhibitor therapy, enabling longitudinal assessment of therapy-induced immunometabolic alterations. In addition to RNAseq, the spatial distribution of cells in the tumor microenvironment will be analysed using tissue microarray and multiplex imaging techniques.

In parallel, wet-lab experiments will be conducted in the Krstić laboratory. These will include functional modulation of AR and ER signaling in appropriate cellular models to investigate downstream effects on metabolic programs and immune-relevant signaling pathways. Basic knowledge of bioinformatics and/or a strong interest in computational data analysis is required for this project.

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Herbert Strobl (PI)/ Theresa Benezeder (Co-PI)

Otto Loewi Research Center (Immunology) and Department of Dermatology, Medical University of Graz

Project Title:
Monocyte differentiation and immune-dysregulation in Ras -associated myeloid diseases

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Tumors harbour macrophage and dendritic cell (DC) subsets that develop from circulating blood precursors in response to microenvironmental signals. Tumor-resident DCs take up dying cells, migrate to draining lymph nodes and present processed antigens to naïve T cells to initiate anti-tumor immune responses. However, tumor cells can also subvert these processes, by instructing DCs to induce antigen-specific T cell tolerance, resulting in tumor immune evasion (via induction of tolerogenic DCs, tol-DCs). The nature of these DC instructive tolerogenic signals remained poorly defined.

Hypothesis and Objectives:

We hypothesize that oncogene-driven constitutive-active MAPK signaling instructs tolerogenic DCs. Ras signaling in hematopopoietic cells can cause monocyte malignancies exhibing certain characteristics of tol-DCs. Moreover, Ras/MAPK signaling in epithelial cells might instruct tumor-resident DCs to adopt tol-DC characteristics via contact-dependent and paracrine factors.

Methodology:

We will perform immunohistology and gene profiling of tumor cells and tumor-resident immune cells. We will utilze differentiation models of human DCs to study their tol-DC programming, and will perform *in vivo* validation studies in the murine system.

References:

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Joanna Szkandera (PI)/ Beate Rinner (Co-PI)

Department of Internal Medicine, Division of Oncology and Diagnostic & Research Center of Pathology, Medical University of Graz

Project Title:

Exploiting Inflammatory-Metabolic Crosstalk for Therapeutic Innovation in Liposarcoma

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Liposarcoma (LPS) is one of the most frequent soft tissue sarcoma subtypes in adults and remains clinically challenging due to high recurrence rates and limited therapeutic options for advanced disease1. Well-differentiated and dedifferentiated liposarcomas are characterized by amplification of chromosome 12q13–15, particularly involving MDM2, yet current systemic therapies provide only modest benefit. Consequently, clinical decision-making continues to rely largely on histopathology and tumor grade, underscoring the need for biologically informed strategies that capture additional dimensions of tumor behavior. Recent studies have revealed a distinctive metabolic vulnerability in liposarcoma, namely a pronounced dependency on extracellular serine to sustain nucleotide biosynthesis, redox homeostasis, and tumor proliferation2. Importantly, this dependency appears to be supported by inflammatory signaling originating from the tumor. Liposarcoma cells have been shown to secrete interleukin-6 (IL-6), which induces metabolic reprogramming in distant skeletal muscle, leading to increased systemic serine availability3. This IL-6–serine axis represents a novel form of tumor–host metabolic cooperation and suggests that liposarcoma growth is not solely determined by tumor-intrinsic metabolic pathways. Cancer is increasingly recognized as a systemic disease that exerts biological effects beyond primary tumor growth and metastasis4. In several malignancies, tumors can hijack host organs to reshape systemic metabolism, thereby creating a permissive environment for disease progression and therapy resistance. Whether the IL-6– serine axis in liposarcoma represents an isolated muscle-specific effect or part of a broader systemic metabolic response remains unknown. Moreover, the therapeutic implications of targeting this axis, either pharmacologically or through metabolic interventions such as nutrient modulation, have not been systematically explored. Understanding how tumor-derived inflammatory signals reshape systemic amino acid metabolism, and how this influences therapeutic vulnerability, represents a critical and unexplored dimension of liposarcoma biology.

Hypothesis and Objectives:

We hypothesize that liposarcoma progression is sustained by an IL-6–driven systemic serine supply network involving multiple host organs, and that tumor-intrinsic factors determine sensitivity to

therapeutic disruption of this axis. The primary objective of this project is to define the biological and therapeutic relevance of the IL-6–serine axis in liposarcoma within a systemic context. Specifically, the project aims to: i.) identify molecular features in primary liposarcoma associated with activation of the IL-6–serine axis, ii.) determine the contribution of tumor-intrinsic regulators to IL-6–mediated metabolic reprogramming using CRISPR-based approaches, and iii.) evaluate therapeutic and nutritional strategies targeting this axis while assessing systemic toxicity.

Methodology:

This project will combine patient-derived tumor material with functional modeling of systemic serine adaptation and therapeutic response. Clinically annotated liposarcoma cohorts will be assembled through an established sarcoma REDCap registry. Primary FFPE tumor specimens will be used to stratify patients according to activation of the IL-6–serine axis using immunohistochemistry, multiplex immunofluorescence, and targeted RNA profiling, enabling correlation with disease progression and outcome. Functional studies will be performed using patient-derived liposarcoma models. Serine adaptation will be modeled dynamically by culturing cells under defined nutrient conditions with controlled serine availability and tumor-conditioned media to mimic systemic metabolic signals. CRISPR-based perturbation of IL-6 signaling components, serine transporters, and key metabolic enzymes will be used to define tumor-intrinsic regulators of metabolic plasticity. Therapeutic efficacy of metabolic and inflammatory pathway inhibitors will be evaluated under serine-adapted and non-adapted conditions to determine how systemic nutrient availability influences drug response. In vivo xenograft and patient-derived xenograft models will be used to assess tumor response alongside circulating metabolite changes. Systemic effects and toxicity will be evaluated using integrated in vivo and iPSC-based organoid platforms, including cardiomyocytes, skeletal muscle, and liver organoids, to define therapeutic windows and distinguish tumor-selective from systemically limiting interventions.

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Armin Zebisch (PI)/ Albert Wölfler (Co-PI)

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Project Title:

Analyzing CBL mutations in acute and chronic myelomonocytic leukemia

*This project is part of the PhD program **TIMO (Translational Immunology and Metabolism in Oncology)**. The program funds 15 positions with clinical/pre-clinical so-supervision (**link**).*

Background:

Acute and chronic myelomonocytic leukemias (AML and CMML) are aggressive hematologic malignancies with poor prognosis, arising from the malignant transformation of hematopoietic stem and progenitor cells (HSPCs). Inactivating *CBL* mutations modify intracellular *RAS*-signaling and frequently contribute to disease development, yet most insights into *CBL*-driven leukemogenesis come from mouse models or immortalized cell lines. Consequently, there is a pressing need for novel models that recapitulate *CBL* inactivation in primary human HSPCs. Within this project, this gap will be addressed in the framework of the doctoral program TIMO (Translational Immunology and Metabolism in Oncology).

Hypothesis and Objectives:

We hypothesize that precise *CBL* inactivation in primary healthy and AML/CMML HSPCs is feasible and will create an unprecedented platform for translational leukemia research. Using this model, we aim to validate pathogenetic and immunological mechanisms of *CBL*-driven leukemogenesis in a realistic and fully human setting. Leveraging the state-of-the-art metabolomics facilities within this PhD program, we will also investigate the role of *CBL* mutations in metabolic reprogramming in AML and CMML. This connection has been proposed but remains largely unexplored due to the lack of suitable human models in the past - our approach is uniquely positioned to address this gap.

Methodology:

The student will use CD34+ HSPC isolated from healthy donors and primary AML/CMML patient specimens to model *CBL* inactivation by CRISPR/Cas9-mediated knock-outs and mutational knock-ins. She/he will also introduce these genetic aberrations by lentiviral shRNA and mutated overexpression vectors. The latter approach will serve as a fail-safe mechanism and enable the immediate start of functional experiments. The student will then use these models to elaborate on the role of *CBL* inactivation in AML and CMML pathogenesis. Therefore, she/he will assess the leukemogenic capacity of *CBL*-edited cells in proliferation and apoptosis assays and assess the effects of *CBL* inactivation on

HSPC differentiation. Thereby, she/he will also profit from the co-supervision of A. Wölfler, who is head of the flow-cytometry unit at the Division of Hematology. Ultimately, the student will also study the effects of *CBL* mutations on metabolome alterations by employing NMR-based metabolomics. Depending on the student's progress, she/he will also have the opportunity to validate these results in a xenotransplantation model, where the genomically engineered human HSPC are transplanted into immunocompromised mice.

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