GEORG SPEYER HAUS
INSTITUT FÜR TUMORBIOLOGIE
UND EXPERIMENTELLE THERAPIE

Zelluläre Kommunikation in der Stammzellnische
Zell-Zell Interaktionen im Tumorstroma
Experimentelle Therapie

Annual Report Georg-Speyer-Haus 2022

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Wissenschaft
und Kunst

Die Grundfinanzierung des Georg-Speyer-Hauses wird vom Bundesministerium für Gesundheit und dem Hessischen Ministerium für Wissenschaft und Kunst getragen.

The basic funding of the Georg-Speyer-Haus is provided by the Federal Ministry of Health and the Ministry of Higher Education, Research and the Arts of the State of Hessen.

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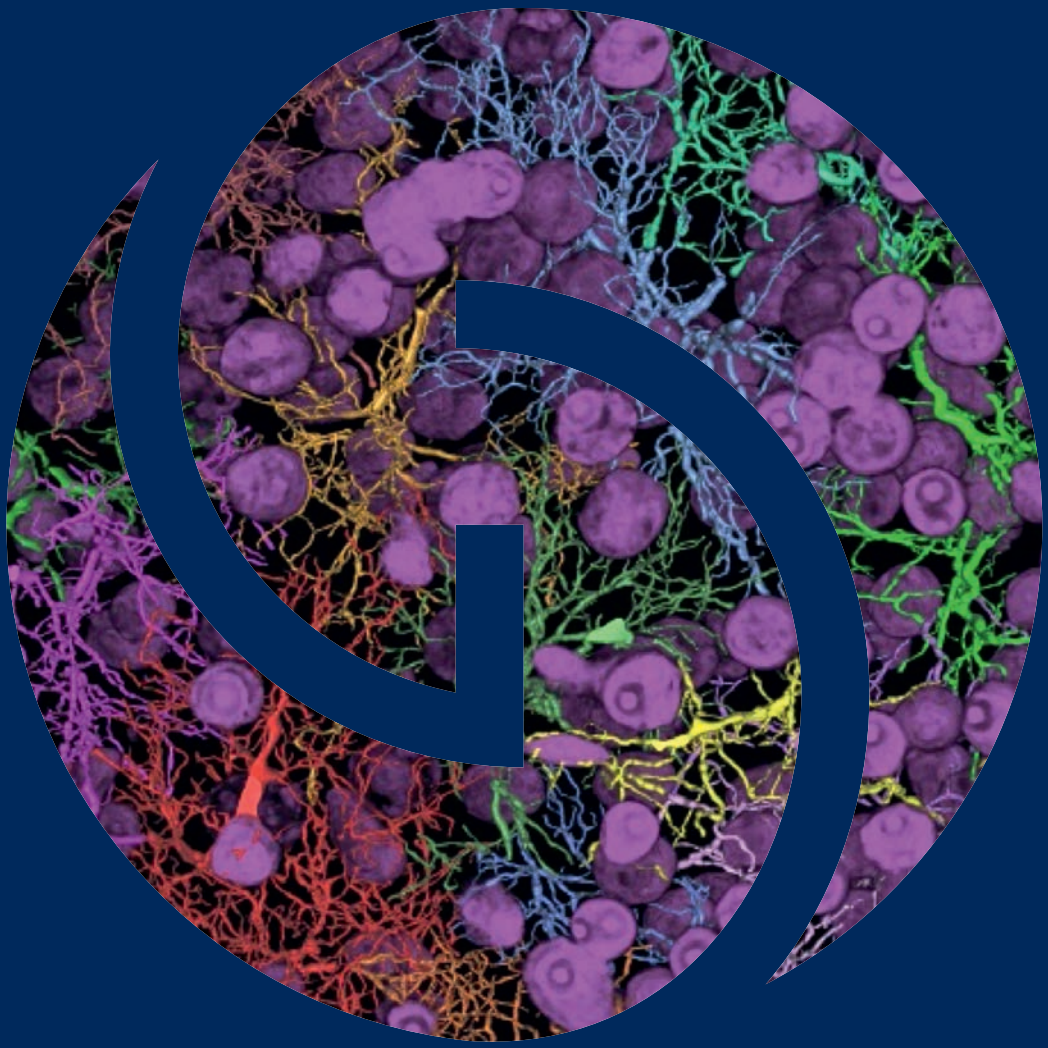

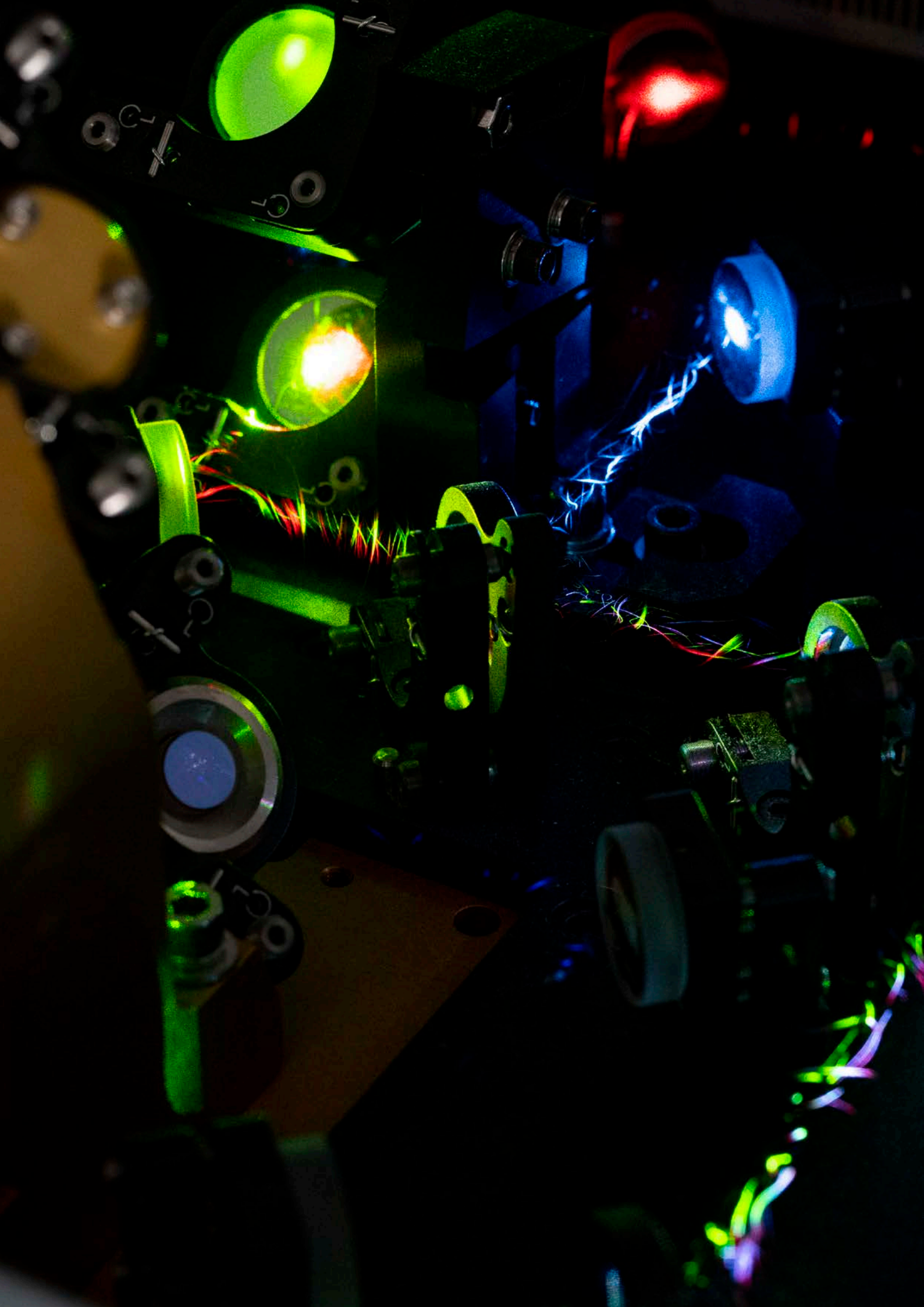

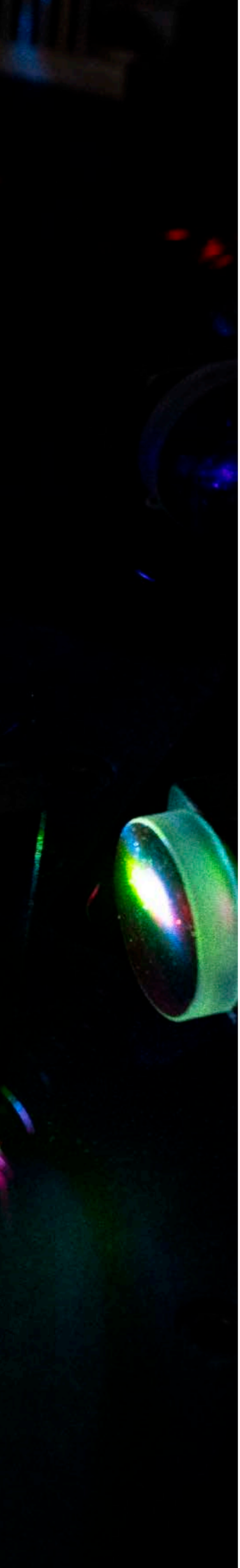

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Liebe Leserinnen und Leser,
liebe Freunde des Georg-Speyer-Hauses,

die Folgen der COVID-Pandemie schränken unsere Tätigkeit inzwischen nur noch marginal ein. Derzeit verzichten wir lediglich noch auf unsere Seminarreihen im Präsenzformat, so dass wir unsere wissenschaftlichen Projekte wieder im Regelbetrieb bearbeiten können.

Allerdings haben auch die Folgen des völkerrechtswidrigen Angriffskriegs Russlands auf die Ukraine bedeutende Auswirkungen auf das Georg-Speyer-Haus. Die unerwarteten und inzwischen massiven Steigerungen der Energiekosten stellen eine außergewöhnliche Herausforderung für uns dar. So rückte die bereits im vergangenen Jahr begonnene Teilnahme an der „Green Lab" Initiative auf einmal zentral in den Fokus und die im Rahmen dieser Initiative vorgeschlagenen Änderungen wurden rasch umgesetzt und umfangreich erweitert, so dass die erste Arbeitsgruppe des Instituts bereits eine Zertifizierung als „Green Lab" erhalten konnte. Zur weiteren Reduktion des Stromverbrauchs wurde der Betrieb des Tierhaltungscontainers auf unserem Grundstück im Sommer eingestellt. Dieser ohnehin immer als temporäre Lösung gedachte Haltungsbereich sollte im Kontext des FCI-Neubaus geschlossen werden und die nun vorgezogene Maßnahme trägt bereits jetzt signifikant zur notwenigen Kostenreduktion bei.
In diesem Zusammenhang möchte ich mich bei unserem Förderverein „Freunde und Förderer des Georg-Speyer-Hauses" aufrichtig bedanken, der es uns ermöglicht hat im Frühjahr kurz nach Ausbruch des Kriegs innerhalb weniger Tage ein Stipendienprogramm ins Leben zu rufen um geflüchtete Wissenschaftler*innen aus der Ukraine bei uns im Haus aufzunehmen und in den ersten Monaten unbürokratisch zu unterstützen. Durch die tatkräftige Hilfe vieler Mitarbeitenden konnten unsere Apartments für diesen Zweck hergerichtet werden.

Dear Reader,
dear friends of the Georg-Speyer-Haus,

The consequences of the COVID pandemic are now only marginally restricting our activities. Currently, we are only refraining from our seminar series in attendance format, so that we run our scientific projects in regular operation again.

However, the consequences of Russia's war of aggression on Ukraine, which is contrary to international law, have also had a significant impact on the Georg-Speyer-Haus. The unexpected and meanwhile massive increases in energy costs represent an extraordinary challenge for us. Thus, participation in the "Green Lab" initiative, which had already begun last year, suddenly came into central focus, and the changes proposed as part of this initiative were quickly implemented and extensively expanded, so that the Institute's first working group was already able to obtain certification as a "Green Lab". To further reduce electricity consumption, the operation of the animal husbandry container on our property was discontinued in summer. This enclosure, which has always been intended to be a temporary solution anyway, was going to be closed in the context of the new FCI building, and the measure that has now been brought forward is already making a significant contribution to the necessary cost reduction.
In this context, I would like to express my sincere gratitude to our sponsoring association "Friends and Supporters of the Georg-Speyer-Haus", which made it possible for us to initiate a scholarship program

Im Sommer konnten wir nach 3 Jahren erstmalig wieder ein gemeinsames Sommerfest ausrichten, das für alle nach einer so langen Zeit ein besonderes Erlebnis war. Lisa Sevenich konnte hier verkünden, dass sie und Stefan Stein vom „GSH Bike & Hike Team“ bei der diesjährigen Sportaktion der Deutschen Krebshilfe vom 13. Mai bis 09. Juni „Gemeinsam gegen Krebs“ die meisten Kilometer sammeln konnten. Auch die vom Betriebsrat organisierte Tombola zur Unterstützung der Deutschen Kinderkrebshilfe wurde ein voller Erfolg und es konnten fantastische Preise und echte „Hingucker“ vergeben werden.

Im Herbst wurde unser mit der Goethe-Universität, dem Paul-Ehrlich Institut in Langen, dem Max-Planck Institut für Herz-und Lungenforschung in Bad Nauheim und zukünftig dem Blutspendedienst gemeinsam betriebenes LOEWE-Zentrum „Frankfurt Cancer Institut“ (FCI) zum ersten Mal zwischenbegutachtet. Wir konnten bei der Begutachtung eindrücklich zeigen, wie erfolgreich die interdisziplinäre Konzeptidee des FCI ist. Daher sind wir optimistisch, dass sich der LOEWE-Programmbeirat der enthusiastischen Empfehlung der Gutachter anschließt und das noch virtuelle Zentrum für weitere drei Jahre fördern wird. Gleichzeitig hoffen wir, dass die Probleme, die zur Verzögerung bei der Planung für den Bau des neuen Gebäudes nun überwunden sind und im nächsten Jahr tatsächlich mit den eigentlichen Bauarbeiten begonnen werden kann.
Auch das nächste Jahr beginnt wieder mit einer Evaluation. Im März erwarten wir den Wissenschaftsrat und wir freuen uns darauf den über die letzten Jahre aufgebauten neuen inhaltlichen Fokus auf das Tumormikromilieu zu präsentieren. Nicht zu letzt auch die vielen strukturellen Änderungen, die neben der inhaltlichen Fokussierung vorgenommen wurden, haben zu einer Vielzahl von erfolgreichen Projekten geführt, über die die folgenden Seiten Ihnen in gewohnter Weise einen Überblick geben sollen.

Florian R. Greten, Direktor

within a few days in spring shortly after the outbreak of the war in order to welcome refugee scientists from Ukraine in our house and to support them unbureaucratically during the first months. Thanks to the energetic help of many employees, our apartments could be prepared for this purpose.

In summer, for the first time in 3 years, we were able to organize a joint summer party, which was a special experience for everyone after such a long time. Lisa Sevenich was able to announce that she and Stefan Stein of the “GSH Bike & Hike Team” were able to collect the most kilometers at this year's sports campaign of the German Cancer Aid from May 13 to June 9 “Together against Cancer”. The raffle organized by the works council in support of the German Children's Cancer Aid was also a great success and fantastic prizes and real “eye-catchers” were awarded.

In fall, our LOEWE center “Frankfurt Cancer Institute” (FCI), which is jointly operated with the Goethe University, the Paul Ehrlich Institute in Langen, the Max Planck Institute for Heart and Lung Research in Bad Nauheim and, in the future, the Blood Donation Service, was subjected to its first interim evaluation. During the review, we were able to impressively demonstrate the success of the FCI's interdisciplinary concept idea. Therefore, we are optimistic that the LOEWE program advisory board will follow the enthusiastic recommendation of the reviewers and fund the still virtual center for another three years. At the same time, we hope that the problems that led to the delay in planning for the construction of the new building have now been overcome and that actual construction work can finally start next year.

Next year will again begin with an evaluation. In March we are expecting the German Science Council and we are looking forward to present the new focus on the tumor microenvironment that has been built up over the last years. Moreover, the many structural changes that have been made in addition to the content focus have led to a large number of successful projects, about which the following pages will give you an overview in the usual way.


CHEMOTHERAPEUTICES
POSTULATUM PRIMUM
UT PROFICIATUR,
PRIMO PROXIMUM
UT NIL NOCEATUR

Forschen für das Leben
Research for Life

Die Stiftung privaten Rechts „Chemotherapeutisches Forschungsinstitut Georg-Speyer-Haus“ wurde 1904 in Frankfurt am Main gegründet, um eine Forschungsstätte für Paul Ehrlich, den ersten Direktor des Hauses, zu schaffen. Die Stiftungsverfassung bestimmt als Zweck der Stiftung die wissenschaftliche Forschung auf den Gebieten der Chemotherapie und verwandter Wissenschaften, die dem Fortschritt der Biomedizin dienen. Es werden ausschließlich und unmittelbar gemeinnützige Zwecke verfolgt.

Die laufenden Geschäfte des heutigen Instituts für Tumorbiologie und experimentelle Therapie nimmt der Direktor wahr. Er ist in dieser Tätigkeit dem Stiftungsvorstand verantwortlich. Das Georg-Speyer-Haus ist durch einen Kooperationsvertrag mit der Goethe-Universität Frankfurt verbunden.

Das Gebäude des Georg-Speyer-Hauses in der Paul-Ehrlich-Straße 42 – 44, 1906 eröffnet, wurde von der Stadt Frankfurt am Main zur Nutzung für Institutszwecke zur Verfügung gestellt. Der gesamte Gebäudekomplex wurde in den Jahren 1995 – 1997 aus Mitteln des Bundesministeriums für Gesundheit und des Hessischen Ministeriums für Wissenschaft und Kunst saniert und modernisiert. Er umfasst eine Gesamtfläche von 4710 qm. Die Laboratorien sind für Arbeiten unter verschiedenen biologischen und gentechnischen Sicherheitsstufen 1 und 2 zugelassen.

The private foundation “Chemotherapeutisches Forschungsinstitut Georg-Speyer-Haus” (Chemotherapeutic Research Institute Georg-Speyer-House) was established in 1904 in order to provide a research institute for Paul Ehrlich, its first director. The constitution of the institute, originating from its foundation, defines its purpose as an establishment for scientific research in the field of chemotherapy and related sciences. It is an independent institution under public law which is exclusively engaged in non-profit work.

Today’s Institute for Tumor Biology and Experimental Therapy is headed by the Scientific Director who reports to the Board of the Foundation. The Georg-Speyer-Haus has a cooperative agreement with the Goethe University Frankfurt.

The Georg-Speyer-Haus is located in a building on Paul-Ehrlich-Str. 42- 44, which has been provided by the City of Frankfurt. The building which was opened in 1906 was renovated in the years from 1995 – 1997 with support from the Federal Ministry of Health and the Ministry of Higher Education, Research and the Arts of the State of Hessen. It comprises an area of 4710 m^2. The laboratories are certified for work under different biological and gene technology safety regulations 1 and 2.

Das Georg-Speyer-Haus wird finanziell vom Bundesministerium für Gesundheit (BMG) sowie vom Hessischen Ministerium für Wissenschaft und Kunst (HMWK) unterstützt. Zusätzlich stehen Mittel aus der Drittmittelförderung öffentlicher und privater Forschungsförderungsorganisationen, aus Kooperationsvereinbarungen mit Unternehmen, aus Erträgen des Stiftungskapitals und aus Spenden zur Verfügung.

Als Partner im Universitären Centrum für Tumorerkrankungen (UCT), dem LOEWE Zentrum für Zell-und Gentherapie (LOEWE-CGT) sowie dem Deutschen Konsortium für translationale Krebsforschung (DKTK) führt das Georg-Speyer-Haus international kompetitive Grundlagenforschung auf dem Gebiet der Tumorbiologie unter besonderer Berücksichtigung des Tumormikromilieus durch. Durch die enge Kollaboration mit den klinischen Partnern der Goethe-Universität im Rahmen der oben genannten Verbünde werden die Ergebnisse aus der Grundlagenforschung in frühe klinische Studien überführt. Darüberhinaus engagiert sich das Georg-Speyer-Haus in der Wissensvermittlung sowie in der Umsetzung neuer Einsichten in therapeutische Applikationen, Dienstleistungen und Produkte und kann so als ein Zentrum der translationalen onkologischen Forschung angesehen werden.

The Georg-Speyer-Haus is supported by the Federal Ministry of Health and the Ministry of Higher Education, Research and the Arts of the State of Hessen. Additional funding is provided by competitive grants, by cooperation agreements with companies, by returns from the investment of the foundation and by private donations.

As a strong partner within the University Cancer Center, the LOEWE Center für Cell and Gene Therapy as well as the German Cancer Consortium the Georg-Speyer-Haus is performing internationally competitive basic research in the field of tumor biology with a particular focus on the tumor microenvironment. In close collaboration with clinical partners at the Goethe-University, results are translated into early clinical trials and the Georg-Speyer-Haus can therefore be considered a center of translational oncology.

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in der Stammzellnische

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und Labor-
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Dr. S. Stein
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Zell-Zell Interaktionen
im Tumorstroma

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Dr. L. Sevenich

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Arbeitssicherheit

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FORSCHUNGSBEREICH 3

Experimentelle Therapie

Prof. Dr. W. Wels

I

Zelluläre Kommunikation in der Stammzellnische

Cellular Communication in the Stem Cell Niche

Die Rolle des Knochenmarksmikromilieus bei den Leukämien

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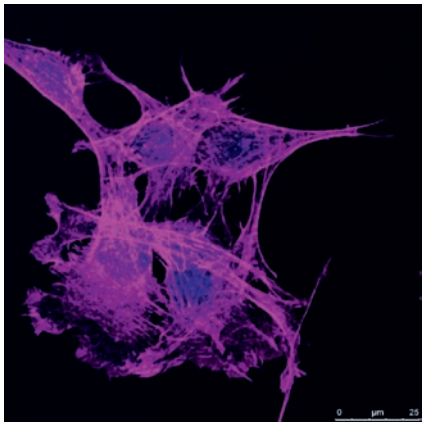
The role of the bone marrow microenvironment in leukaemia

leukaemia

bone marrow microenvironment

pharmacological modulation

The bone marrow microenvironment (BMM) is increasingly being considered as a novel target to augment existing therapies for haematological malignancies. This is important, as the overall survival rate for all leukaemias in adults is only 44%, and leukaemic stem cells (LSC) are rarely eradicated. Eradication of cancer stem cells in leukaemia or LSC, however, is thought to be important for cure of a cancer.

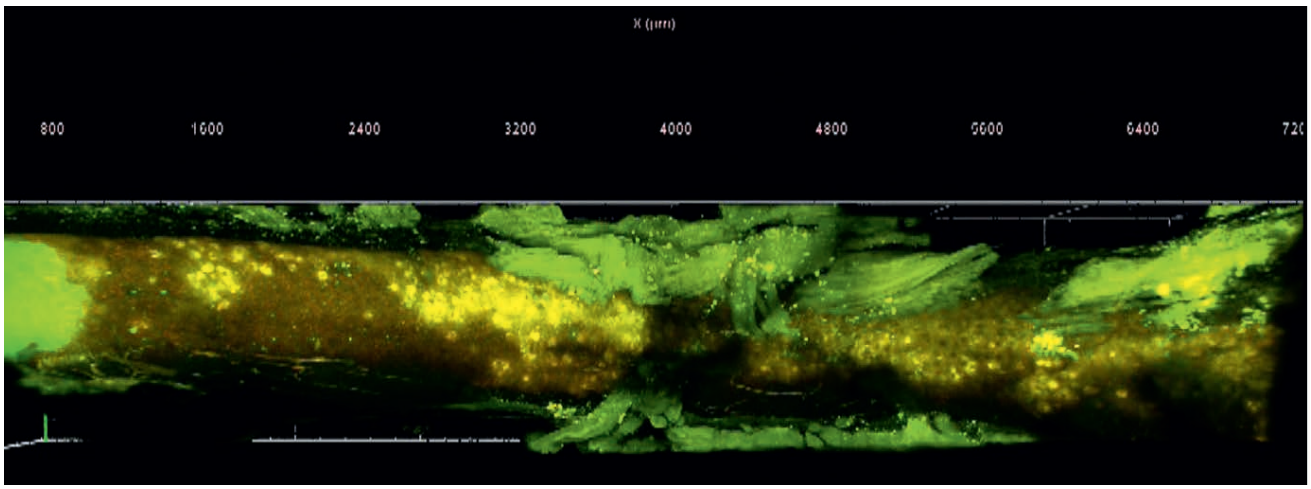


Immunofluorescence studies on 3T3 fibroblasts grown on coverslips and stained with phalloidin (F-actin). The nucleus is stained with DAPI (blue), and F-actin is stained with AF-647 (magenta).

Trotz verbesserter Therapien, z.B. in Form von Medikamenten aus der Gruppe der personalisierten Medizin, liegt die 5-Jahres-Überlebensrate bei Erwachsenen für alle Leukämien bei nur 40%. Deshalb hat es sich unsere Arbeitsgruppe zur Aufgabe gemacht, neue Therapien, vor allem solche mit neuem Therapieansatz, zu entwickeln.

Eine gezielte Modulation des Knochenmarksmikromilieus (KMMM), dem Ort, wo eine Leukämie in der Regel entsteht und voranschreitet, kann eine Verringerung von leukämischen Stammzellen nach sich ziehen. Dies ist notwendig, denn leukämische Stammzellen können zu Therapieresistenz und Krankheitsrückfall führen. Das KMMM, welches leukämische Stammzellen vor der Chemotherapie „beschützen" kann, besteht aus verschiedenen Zelltypen wie Osteoblasten, Osteoklasten, mesenchymalen Stammzellen, Endothelzellen, und der extrazellulären Matrix.

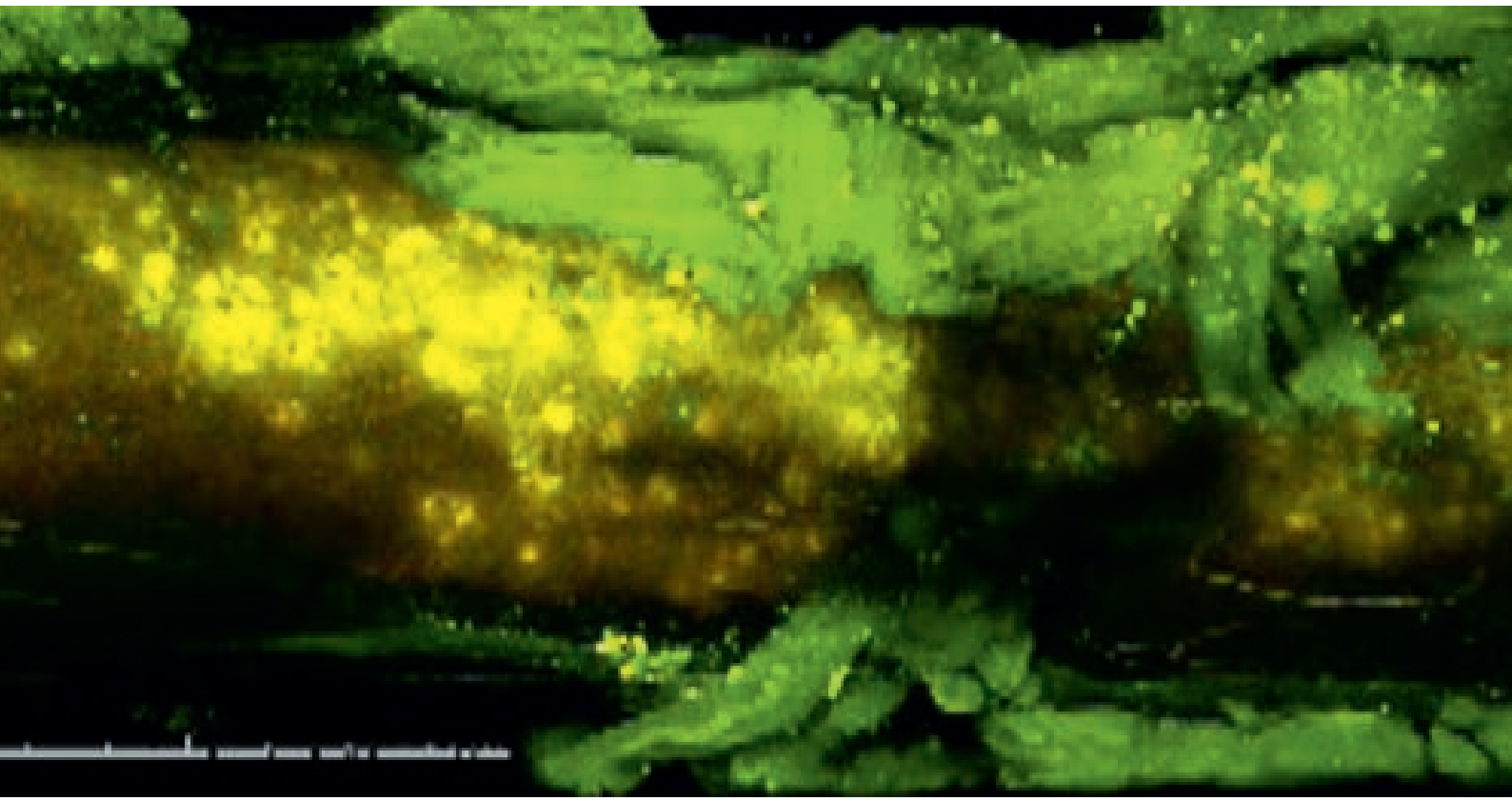
Wir haben gezeigt, dass spezifische Interaktionen von Leukämiezellen mit verschiedenen zellulären und azellulären Komponenten des KMMMs oder das Alter des KMMMs spezifisch den Krankheitsverlauf einer Leukämie beeinflussen können. Ferner sind chemische und metabolische Faktoren im KMMM und qualitätskontrollierende Prozesse in den Nischenzellen des KMMMs, sowie Entzündung im KMMM der Fokus unserer Arbeitsgruppe.



Maximum intensity projection (MIP) fluorescence image of non-sectioned cleared bone imaged using confocal microscopy. Transplanted GFP-labelled cells can be observed in the bone marrow cavity in yellow.

Based on our previous work our laboratory focuses on various pathways of interaction of leukaemia cells with their surrounding bone marrow microenvironment in an effort to eventually target these interactions and eradicate LSC. The extracellular matrix, the coagulation system, chemical factors and novel pathways of adhesion to the BMM, studied by various in vitro and *in vivo* modelling systems, as well as *in vivo* 2-photon based imaging, hereby, form the basis of our studies.

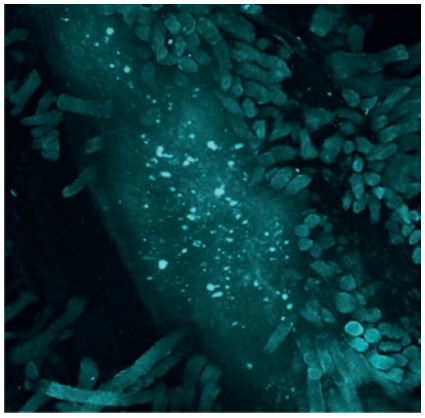
We studied the influence of the age of the bone marrow microenvironment on leukaemia progression. This is important, as B-cell acute lymphoblastic leukaemia (B-ALL) occurs most commonly in children, while chronic myeloid leukaemia (CML) is more frequent in adults. The myeloid bias of haematopoiesis in elderly individuals has been considered causative of these differences, but the age of the BMM is contributory. In fact, our study has shown that the age of the BMM influences the leukaemia phenotype, at least partially via a certain cytokine, CXCL13, and its respective receptor, CXCR5. We demonstrated that high expression of CXCR5 may predict central nervous system relapse in paediatric B-ALL, offering novel avenues for treatment.



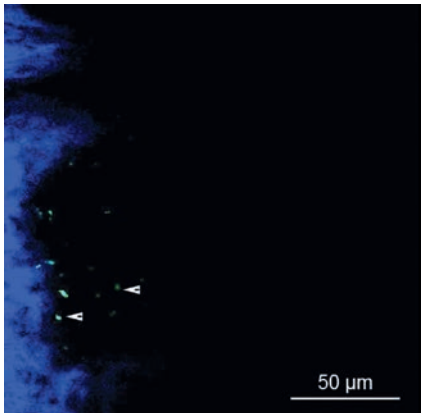
In another project we found that lipid raft-associated molecules play a prominent role for the engraftment of leukaemia cells, possibly via association with the adhesion molecule CD44. In addition, we unraveled how a leukaemia conditions its microenvironment, leading to the secretion of extracellular vesicles from stroma cells and, thereby, in turn the modulation of leukaemia progression. Other projects in the laboratory are involved with quality control pathways in bone marrow niche cells, as well as inflammation and chemical factors influencing leukaemia outcome.

Given the current pandemic due to SARS-CoV-2, we have recently submitted a manuscript focusing on genetic determinants in the clinical course of infection with SARS-CoV-2 in collaboration with the German Red Cross and the Departments of Infectious Diseases, Virology and Bioinformatics at the Goethe University, funded by the Goethe University's Corona-Funds.

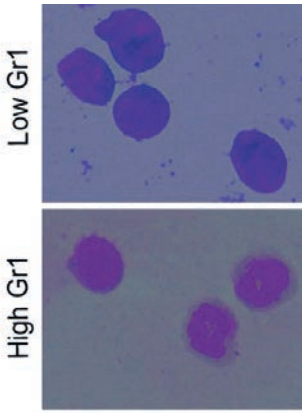
In summary, the laboratory focuses on the role of the different constituents of the BMM on the initiation, maintenance and progression of leukaemias in an attempt to develop novel therapies which can augment our existing armamentarium against this intractable disease. Two ongoing projects have led to the discovery of innovative ways



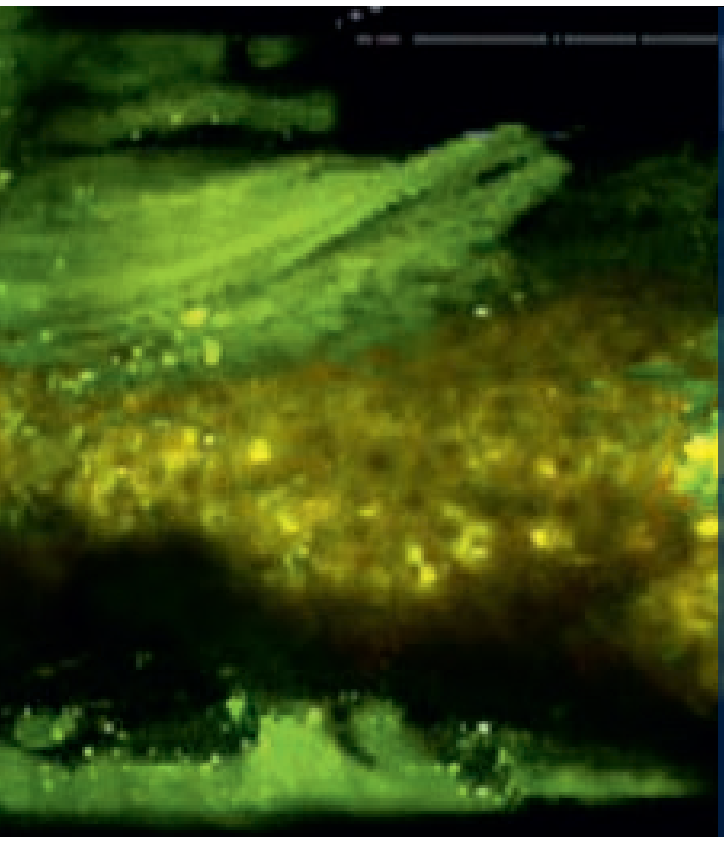
Maximum intensity projection (MIP) fluorescence image of non-sectioned cleared bones imaged using confocal microscopy. Transplanted GFP-labelled cells can be observed in the bone marrow cavity in bright green.



Representative two-photon intravital microscopy image of the calvarium of a mouse, which had been transplanted with GFP-expressing cells. Cells fluoresce green as indicated by the arrows, and the bone fluoresces blue.



Representative Giemsa staining of leukocytes isolated from the bone marrow of mice with acute myeloid leukemia with low or high surface expression of Gr1.



Ausgewählte Publikationen

Karantanou C, Minciacchi VR, Kumar R, Zanetti C, Bravo J, Pereira RS, Tascher G, Tertel T, Covvarubias-Pinto A, Bankov K, Pfeffermann L-M, Bonig H, Divieti-Pajevic P, McEwan DG, Giebel B, Münch C, Dikic I, Krause DS. *Impact of mesenchymal stromal cell-derived exosomal cargo on B-cell acute lymphoblastic leukemia progression. Blood Adv. 2022 Aug 31:bloodadvances.2022007528. doi: 10.1182/bloodadvances.2022007528. Online ahead of print.*

Kumar R, Pereira RS, Niemann J, Azimpour AI, Zanetti C, Karantanou C, Minka W, Minciacchi VR, Kowarz E, Meister M, Godavarthy PS, Maguer-Satta V, Lefort S, Wiercinska E, Bonig H, Marschalek R, Krause DS. *The differential role of the lipid raft-associated protein flotillin 2 for progression of myeloid leukemia. Blood Adv. 2022 Jun 28;6(12):3611-3624.*

Zanetti C, Kumar R, Ender J, Godavarthy PS, Hartmann M, Hey J, Breuer K, Weissenberger ES, Minciacchi V, Karantanou C, Gu Z, Roberts KG, Metzler M, Stock W, Mullighan CG, Bloomfield CD, Filmann N, Bankov K, Hartmann S, Hasserjian RP, Cousins A, Halsey C, Plass C, Lipka DB, Krause DS. *The age of the bone marrow microenvironment influences B-cell acute lymphoblastic leukemia progression via CXCR5-CXCL3. Blood. 2021;138(19):1870-1884*

Kumar R, Pereira R, Zanetti C, Minciacchi VR, Merten M, Meister M, Niemann J, Dietz MS, Rüssel N, Schnütgen F, Tamai M, Akahane K, Inukai T, Oellerich T, Kvasnicka HM, Pfeifer H, Nicolini FE, Heilemann M, Van Etten RA, Krause DS. *Specific, targetable interactions with the microenvironment influence imatinib-resistant chronic myeloid leukemia. Leukemia. 2020, 34(8):2087-2101*

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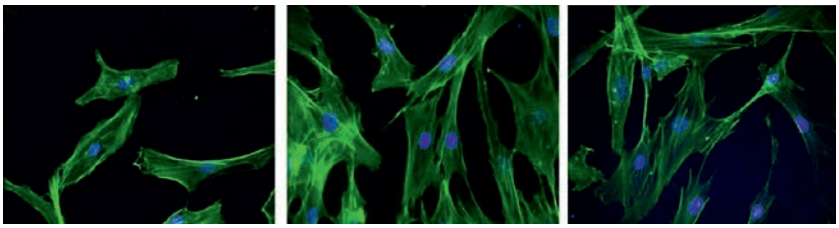
finden Sie auf Seite ▶ 55

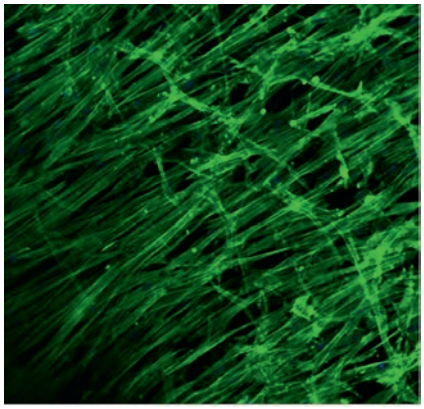
of targeting the BMM, which are to be tested in clinical trials in future.

Other activities

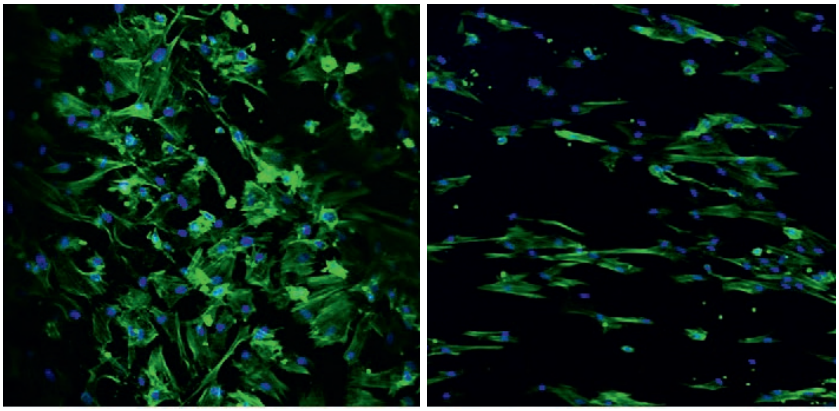
We are also coorganizing the 24th international scientific meeting on "Chronic myeloid leukaemia" under the umbrella of the European School of Haematology. We are actively collaborating with pharma on research involved in the leukaemic bone marrow microenvironment.



Representative immunofluorescence images of human breast fibroblasts (CCD-1069SK) embedded for 24h in different concentrations of collagen, from left to right: 0.25 mg/mL, 2mg/mL and 4 mg/mL. The nucleus is stained with DAPI (blue), and F-actin is stained with AF-488 (green).



Representative immunofluorescence image of human colorectal cancer fibroblasts seeded in PLGA scaffolds for 1 week. The nucleus is stained with DAPI (blue), and F-actin is stained with AF-488 (green).



Representative immunofluorescence images of human astrocytes seeded in PCL (left) and PLGA (right) scaffolds for 48h. The nucleus is stained with DAPI (blue), and F-actin is stained with AF-488 (green).

Tumornischen Evolution

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Tumor niche evolution

Tumor microenvironment

Metastasis/Leukemia

Aging

Overview

Our understanding of cancer has evolved from a tumor cell centric towards a comprehensive view that incorporates extrinsic inputs from the surrounding tumor microenvironment (TME). The TME is a complex ecosystem in which multi-directional crosstalk between cancer cells and a plethora of surrounding stromal cells shape a local milieu that favors the acquisition of the "hallmarks of cancer", such as the ability to evade immune control or metabolic adaptation that facilitates effective colonization of distant organs[1]. Notably, although they support the acquisition of common cancer traits, TMEs are heterogeneous (e.g. depending on tumor type or organ site) and dynamic (e.g. depending on disease stage or patient age), both in terms of their cellular make up and molecular priming. This implies the need to define niche-dependencies in a time and organ-site resolved manner and adapt niche-directed therapeutic strategies accordingly.

Our team devotes substantial effort to *(1) dissect the role of the TME in tumor immune evasion* and *(2) explore the contribution of age-related niche changes to cancer progression and therapy resistance,* across different cancer types. To do so, we develop cutting-edge models and use state-of-the-art technologies, including genetically engineered mouse models, fully human 3D model systems as well as multiplex imaging and single-cell omics approaches.

Project Highlights

Lifting immune suppression to promote cancer control

Cancer is associated with an immune suppressive TME that limits anti-tumor immunity and hampers responsiveness to adaptive immune checkpoint blockade (ICB; anti-PD1/PDL1; anti-CTLA4. etc.). This promotes immune evasion and therapy resistance, respectively. Our goal is to unravel the mechanisms at play to devise new strategies that could lift immune suppression, boost anti-tumor immunity, and elicit susceptibility to ICB thereby enabling lasting therapeutic benefits. We do so in the context of leukemias and brain metastasis, both of which are poorly responsive to systemic immunotherapies using ICB.

Unser Ziel ist es, ein umfassendes Verständnis von äußeren Einflüssen und molekularen Signalwegen zu erlangen, die zum Fortschreiten von Krebs bei malignen hämatologischen Erkrankungen als auch bei der Metastasierung solider Tumore eine Rolle spielen. Wir fokussieren uns auf die Entschlüsselung der Mechanismen der Zell-Zell-Kommunikation, welche (i) die Umgehung der antitumorale Immunität ermöglichen und die (ii) der Krebsprogression im Alter zugrunde liegen. Den Schwerpunkt legen wir hier auf die Rolle der Tumormikroumgebung. Um dies zu untersuchen, verwenden wir modernste genetische Mausmodelle, künstlich hergestellte humane organotypische 3D-Systeme, sowie von Patienten stammende Explantatkulturen und Xenotransplantationsmodelle.

Für die Analyse dieser Modelle verwenden wir modernste Multi-omics-Ansätze. Um unsere Fragen multidisziplinär anzugehen, nutzen wir öffentlich zugängliche Datensätze und arbeiten intensiv mit unserem Netzwerk von Kooperationspartnern zusammen. Unsere Arbeit wird grundlegende Mechanismen entschlüsseln, die der krebsfördernden Eigenschaft der Mikroumgebung von Tumoren zugrunde liegt, und sie wird die Entwicklung neuer und spezifischerer therapeutischer Strategien ermöglichen.

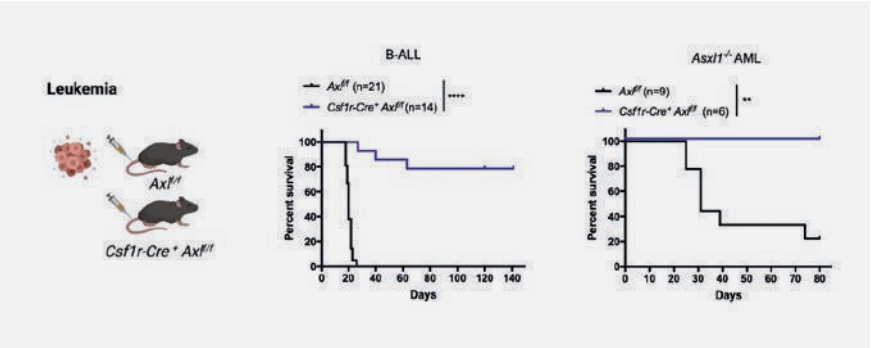


Figure 1. **AXL ablation in macrophages elicits leukemic clearance.** Kaplan-Meier survival of mice with AXL proficient ($Axl^{f/f}$) or AXL deficient (Csf1r-Cre+ $Axl^{f/f}$) macrophages, challenged with Philadelphia-chromosome positive B-cell acute lymphoblastic leukemia (B-ALL) or acute myeloid leukemia driven by Asxl1 deficiency ($Asxl1^{-/-}$ AML).

Acute leukemias are aggressive blood cancers that excel at immune evasion. Due to their low mutational load (i.e. few neo-antigens), leukemias were initially thought to passively escape immune control. Work from our group, recently discovered that leukemia cells actively co-opt macrophages to activate a signaling axis, namely the GAS6/AXL axis, that puts the break on anti-leukemic immunity by hampering the early steps of the "cancer immunity cycle"[2] thus driving the establishment of a suppressive TME. Notably, we demonstrate that targeting AXL, via pharmacological inhibition or genetic ablation in macrophages, not only lifts the barriers towards effective anti-leukemic immunity (Fig. 1) but also elicits susceptibility to ICB[3]. Ongoing efforts are directed towards clinical translation and assessment of other candidate suppressive mediators in this context.
In the solid cancer arena, we are tackling the challenge posed by brain metastasis (BrM), which represent the most frequent intra-cranial tumor in adults and is associated with a dire outcome. BrM incidence is steadily increasing due to better control of extracranial disease and a higher number of cancer survivors at risk of presenting with BrM. On a cellular level, BrM lesions show a massive recruitment of suppressive myeloid cells which together with other brain resident cells (e.g. glial cells) enforce the establishment of an immune-suppressive TME[4] (Fig. 2). Although immune checkpoint blockers (ICB) have been reported to provide clinical benefit in a small fraction

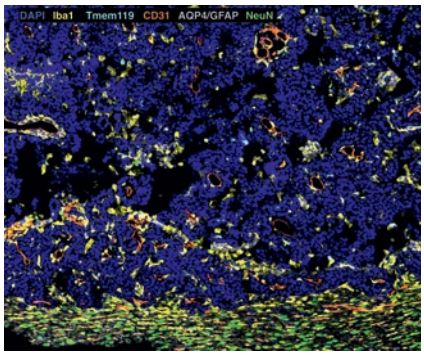
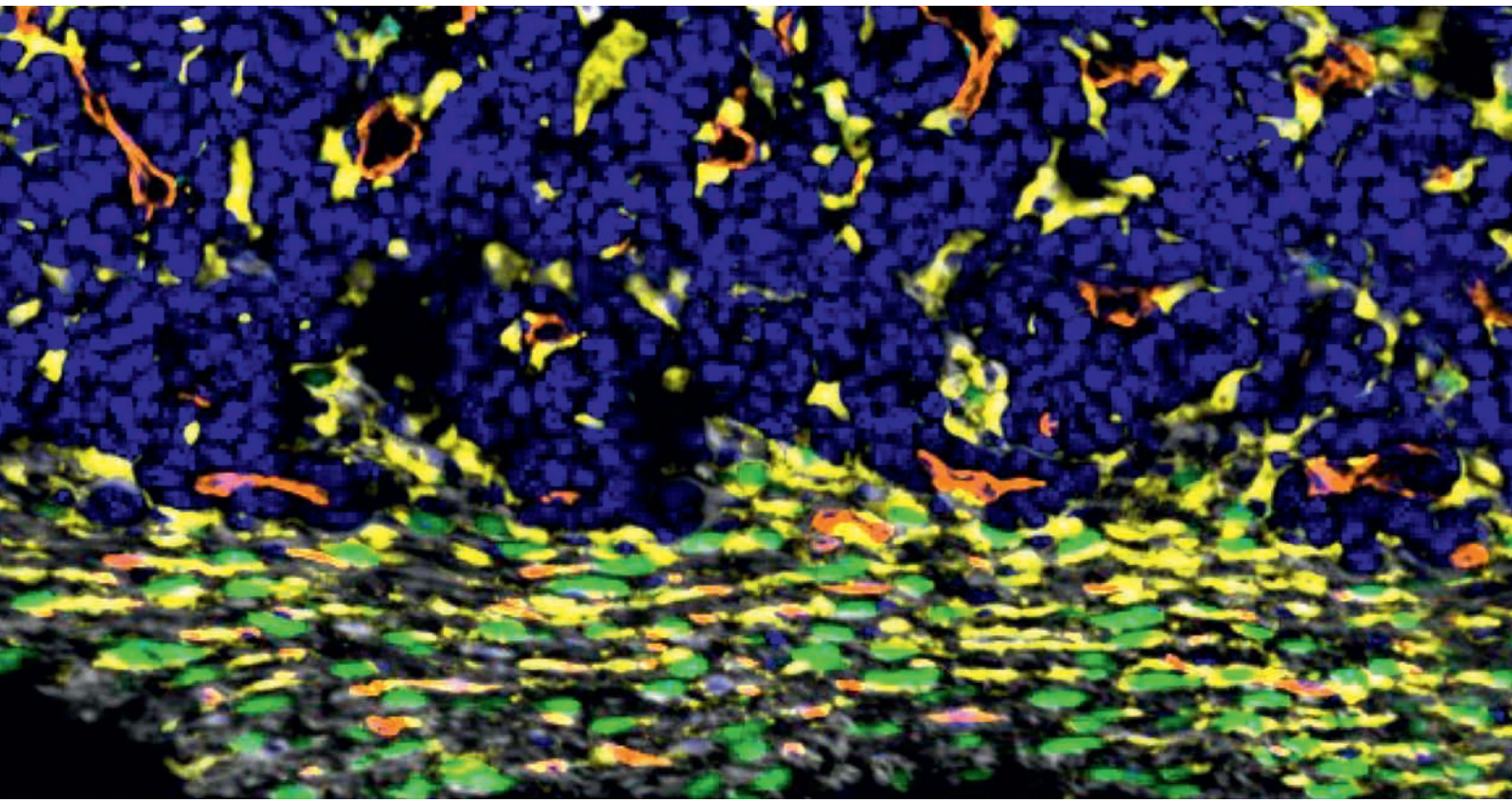


Figure 2. **Cellular architecture of a breast-to-brain metastasis visualized by multispectral imaging.**
Astrocytes are marked by GFAP and Aquaporine 4; Endothelial cells are marked by CD31; Myeloid cells are marked by Iba1; Microglia [the brain resident macrophages] are marked by Tmem119; Neurons are marked by NeuN; Nuclei marked by DAPI.



of patients, more limited effects are seen in advanced disease[4]. We hypothesize that lifting immune suppression locally in the brain, is a pre-requisite to boost anti-tumor immunity and achieve effective immunotherapies in BrM, a task we currently tackle using a holistic and interdisciplinary approach in the context of a team-science project, RISEBrain, supported by the European TRANSCAN-3 funding scheme (https://transcan.eu/output-results/funded-projects/risebrain.kl). This joint venture enables us to put together our expertise in engineering, imaging, computation, experimental modeling, and clinical care, to uncover and target cellular and molecular drivers of local immune suppression in BrM.

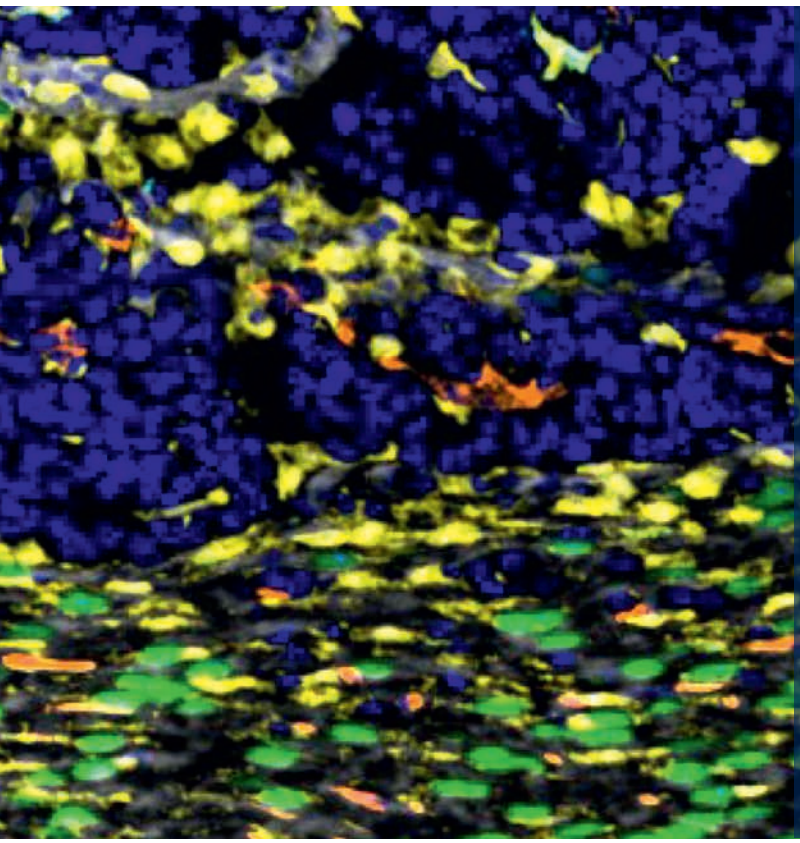
Impact of age-related niche changes on cancer

Physiological aging is accompanied by a progressive decline in health and tissue/organ function as well as a significant increase in cancer risk. The latter represents a tremendous societal and economic challenge in our aging society. In our lab, we study *how age-related changes in the bone marrow (BM) microenvironment* (cellular architecture, composition, and molecular priming) *impact cancer development/progression in the bone*. We do so in the context of hematological malignancies and solid tumor metastasis to the bone.

In solid cancer, we focus on breast cancer because it exhibits high tropism to the bone and displays long periods of tumor dormancy, that can last up to decades, before the development of overt metastasis leading to poor quality of life and patient death. Cues leading to the activation of these dormant disseminated tumor cells (DTCs) remain poorly understood, but advanced age is one of the most significant predictors of overt bone metastasis occurrence. As part of the µBone consortium (https://www.microbone.de) we explore *how age-related alterations in the BM niche drive the awakening of quiescent metastatic cancer cells* to propose new means by which we could improve outcome and/or quality of life of patients with bone metastasis.

In hematological malignancies, we focus on Myelodysplastic Syndromes (MDS)[5,6], a group of syndromes that are characterized by ineffective hematopoiesis with peripheral cytopenia, as well as its precursor state, referred to as clonal hematopoiesis of indeterminate potential (CHIP). We interrogate whether age-related niche changes contribute to the progressive clonal dominance observed in CHIP and MDS as well as the cellular and molecular mediators underlying this phenotype. This project builds on our previous finding that disease-associated mesenchymal niche cells are essential for MDS maintenance[7] and is part of the MDS-INTERCEPT Innovative Training Network (https://intercept-mds.eu) that brings together 10 European public and private institutions with expertise in leukemia, epigenetics and single-cell approaches to promote early disease interception in the context of clonal myeloid diseases.

Experimentally, both research directions are supported by patient-derived xenografts in genetically engineered models with niche alterations that recapitulate those associated with physiological aging[8].



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*equal contribution

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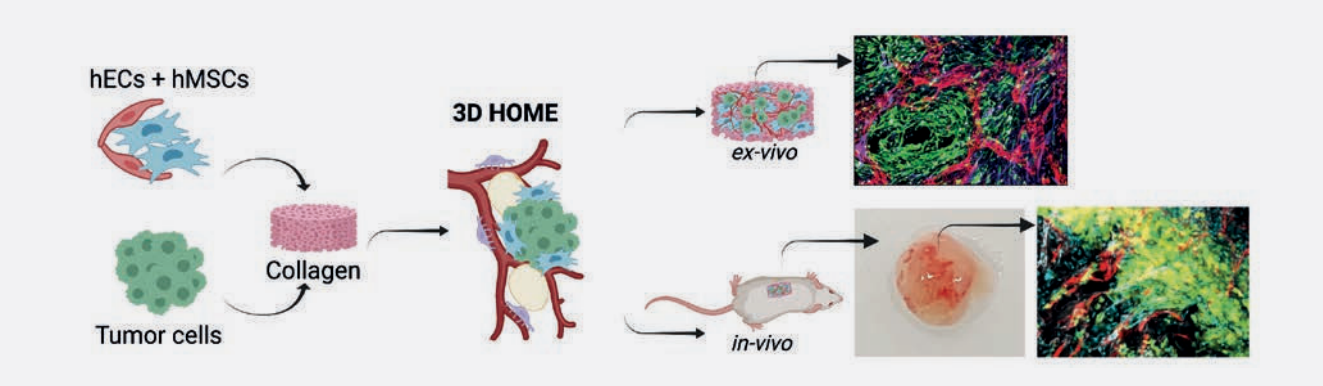

Figure 3. **Schematic view of the 3D HOMEs used to study reciprocal crosstalk between tumor cells and the bone marrow microenvironment.** Representative images show tumor cells in green (GFP+), the vasculature (Red) and stromal cells of mesenchymal origin (Cyan or purple). hEC= human endothelial cells. hMSC= human mesenchymal stromal cells.

This is complemented by newly developed, highly modular and versatile 3D Human Organotypic Marrow Environments (3D HOMEs), that are easily amenable to experimental manipulation (visualization, CRISPR-editing, drug treatment) and in which malignant cells are studied in a fully human setting that closely recapitulate the cellular composition and architecture found in the human bone marrow (Fig. 3).

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GFP
Guest
Find & Focus
LIGHT
ON
14%
life

II

Zell-Zell Interaktionen im Tumorstroma

Cell-Cell Interaction in the Tumor Stroma

Diät, Energiemetabolismus und Krebs

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Diet, Energy Metabolism, and Cancer

Metabolic Derangements during Cancer

Diet, Microbiome, and Cancer

Modeling Host-Microbiome Interactions *in vitro*

Diet is shaped by multiple diverse factors such as culture, nutritional knowledge, price, availability, taste, and convenience. Because diet is one of the major determinants in shaping gut microbiome, which majorly contributes to regulation of metabolic functions and immune homeostasis, reciprocal interactions between host, microbiome, and environmental factors are critical during health. Due to the distinct shifts in agriculture and changes in crops in the last decades, nutrition plays a pivotal role in aggravating disease. Our research aims at delineating how changing diet is associated with cancer initiation and progression in pancreas and intestine at a molecular and cellular level. Using clinical samples and preclinical models, we aim at identifying not only host changes in metabolism but also derangements in microbial interactions. Because host-microbial interactions could be targeted during disease progression, we investigate whether dietary interventions may eventually pave the way for individual-based therapy.

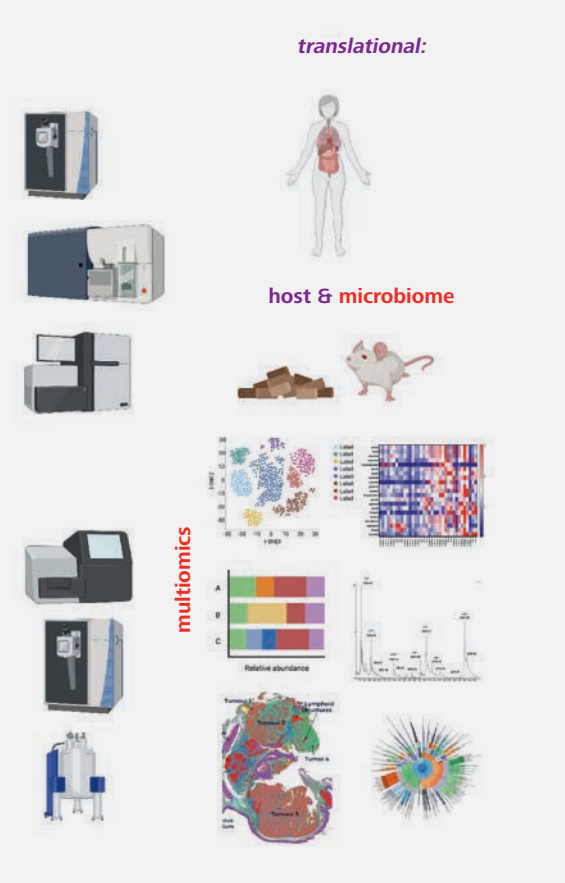


Figure 1.
Metabolic alterations during disease and therapy are investigated using clinical samples and preclinical models with the technology platforms established.

Die Ernährung wird von mehreren unterschiedlichen Faktoren wie Kultur, Ernährungswissen, Preis, Verfügbarkeit, Geschmack und Bequemlichkeit beeinflusst. Da die Ernährung eine der wichtigsten Determinanten für die Gestaltung des Darmmikrobioms ist, das wesentlich zur Regulierung der Stoffwechselfunktionen und der Immunhomöostase beiträgt, sind die Wechselwirkungen zwischen Host, Mikrobiom und Umweltfaktoren für die Gesundheit entscheidend. Aufgrund des deutlichen Wandels in der Landwirtschaft und die Veränderungen bei den Nutzpflanzen in den letzten Jahrzehnten, spielt die Ernährung eine entscheidende Rolle bei der Verschlimmerung von Krankheiten. Unsere Forschung zielt darauf ab, herauszufinden, wie eine veränderte Ernährung mit der Entstehung und dem Fortschreiten von Krebs in der Bauchspeicheldrüse und im Darm auf molekularer und zellulärer Ebene verbunden ist. Mit Hilfe von klinischen Proben und präklinischen Modellen wollen wir nicht nur Veränderungen im Stoffwechsel des Wirtes, sondern auch Störungen der mikrobiellen Interaktionen identifizieren. Da die Interaktionen zwischen Host und Mikroorganismen während des Krankheitsverlaufs gezielt beeinflusst werden könnten, untersuchen wir, ob Ernährungsinterventionen möglicherweise den Weg für eine individuelle Therapie öffnen könnten.

Metabolic Derangements during Cancer

Cancer is marked by dysregulation of signaling pathways that orchestrate proliferation, cell death, tumor-promoting inflammation and energy metabolism. Using mouse- or patient-derived organoids as *in vitro* disease modeling system (Fig. 2a), our studies focus on defining key transcriptomic, proteomic and metabolomic profiles, comparing relevance to primary tumor tissue, and eventually utilizing as a tool system to target metabolic vulnerabilities during cytotoxic therapy, which may have a prognostic value in pancreatic and intestinal cancer. To elucidate, first we identified metabolic alterations in tumor-derived organoids and cells of the tumor microenvironment (TME) (Fig. 2b). Based on this knowledge, then we challenged the metabolic vulnerability using compounds *in vitro* (Fig. 2c-d). Indeed, treating tumor-prone mice with a compound, that could switch metabolic dependency of TME, significantly halted disease progression *in vivo*, too (Fig. 2e).

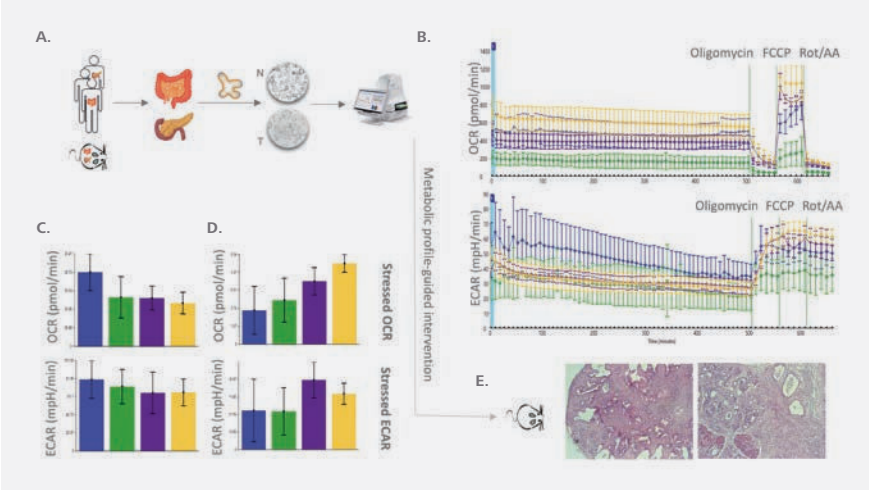
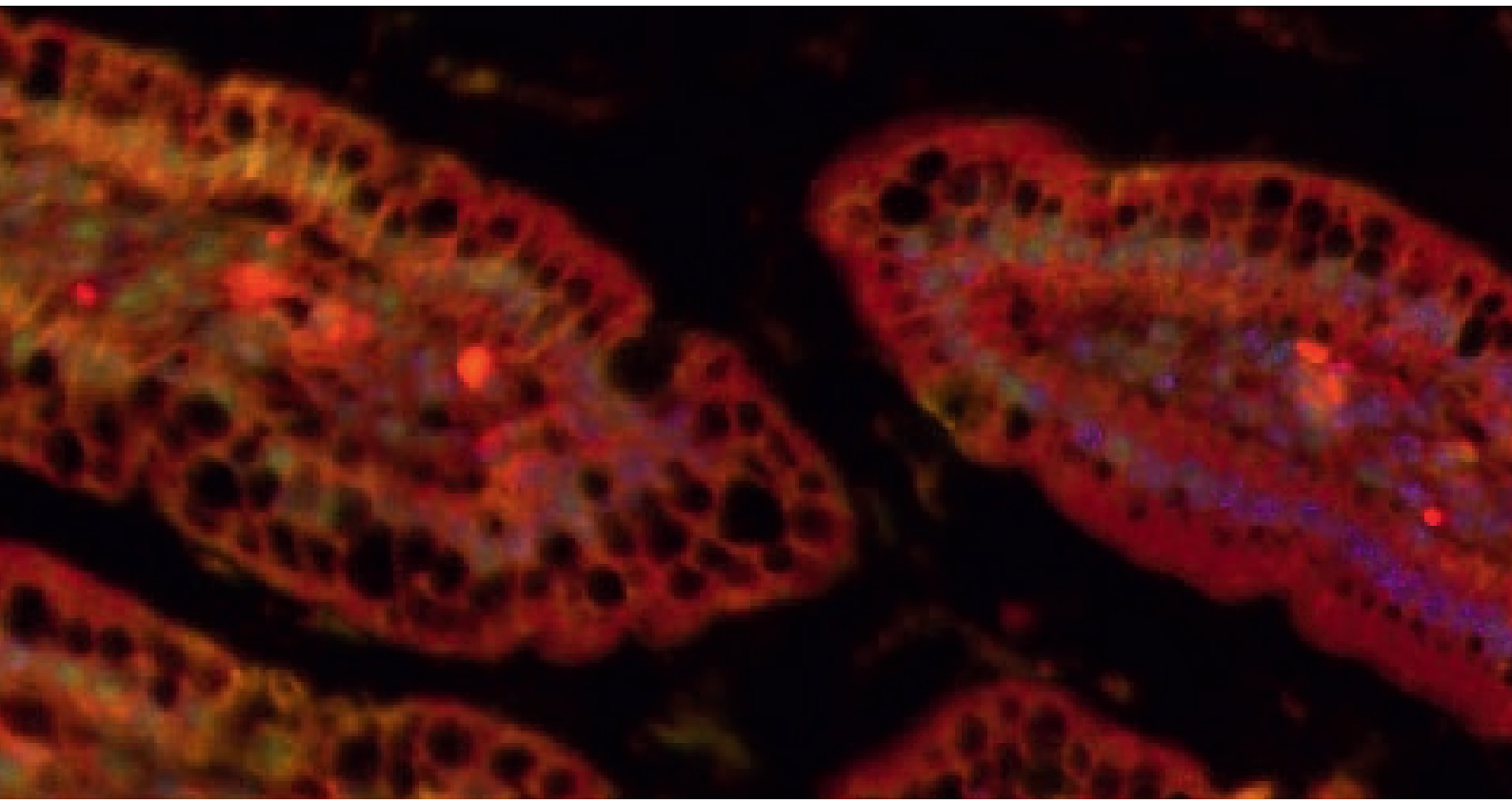


Figure 2.
Using preclinical models, derangements in bioenergetic pathways during disease and therapy are investigated in order to define and target vulnerabilities, which may set the stage for future drug discovery for therapeutic interventions.



Diet, Microbiome, and Cancer

Human gut is inhabited by billions of bacteria contributing majorly to the regulation of metabolic functions and immune homeostasis. Because microbiota composition and function shape susceptibility to cancer and treatment efficacy, determining dynamics of bacterial community in patients undergoing therapy is critical. To help address whether specific bacterial community structure may have diagnostic and prognostic value, we identified disease- or therapy-induced microbial community changes in patient cohorts (Fig. 3a). Bacterial richness, evenness, and diversity changed significantly upon treatment (Fig. 3b-c). We identified the relative abundance of bacteria and predictive signatures in good and poor responders upon cytotoxic therapy (Fig. 3d). Diet can directly or indirectly modulate microbiome and play a decisive role in disease outcome. Using preclinical mouse models, our studies aim at unravelling the impact of varying dietary nutrients on microbiota structure and function during cancer and therapy response. We elucidate whether precision nutrition can pave the way for individual-based interventions in cancer by regulating microbiome and bacterial metabolism.

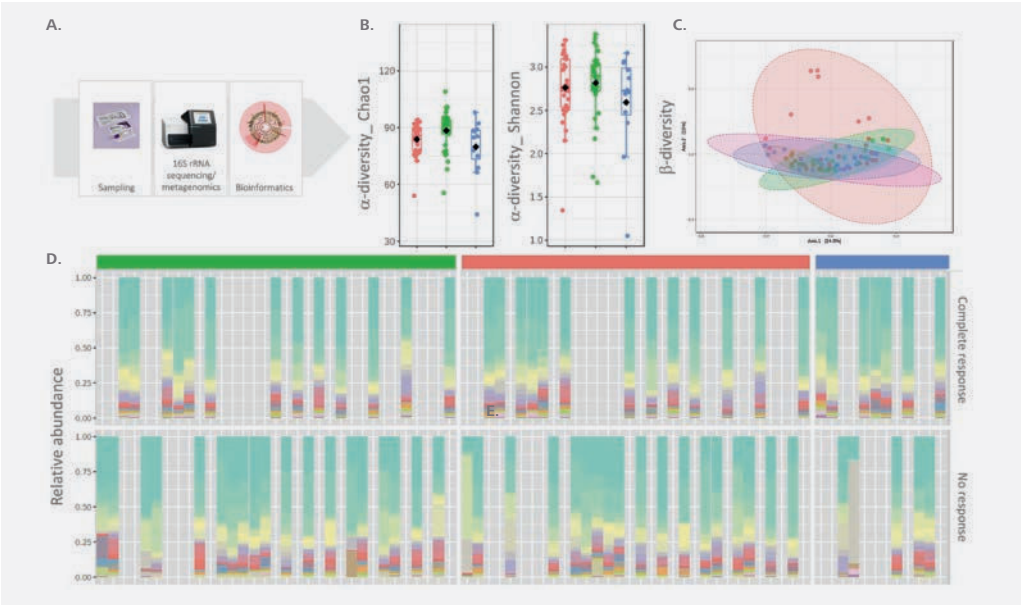
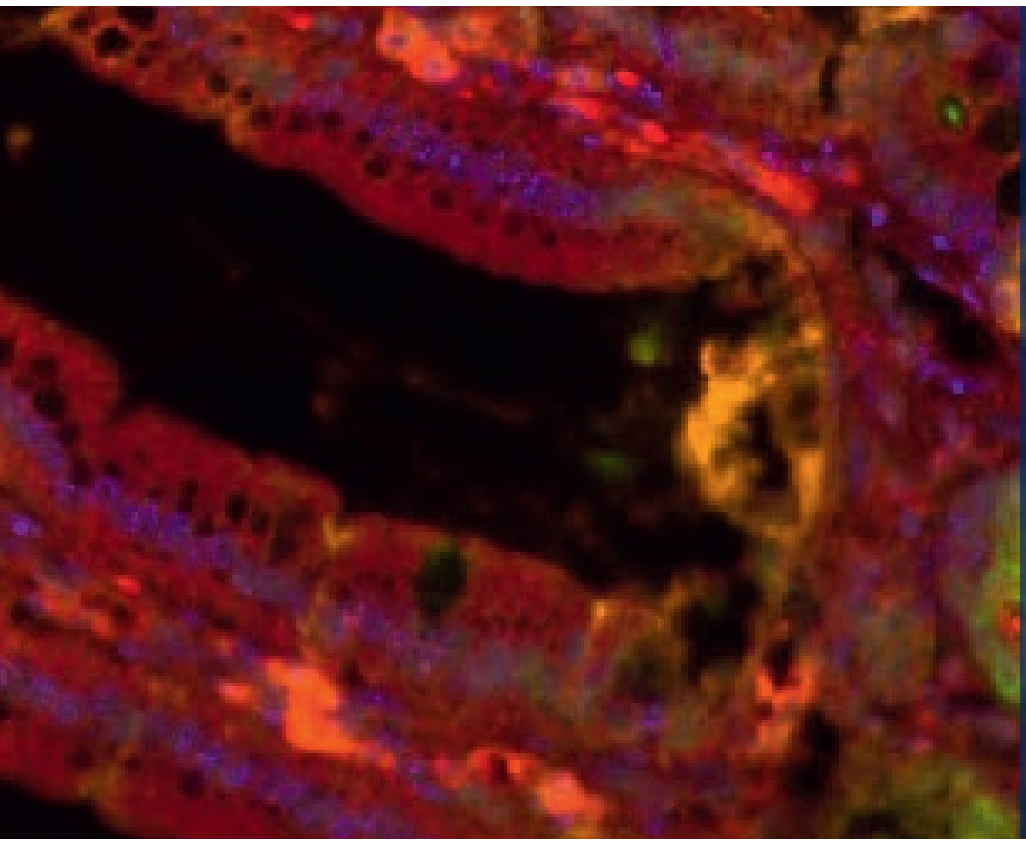


Figure 3.
Defining alterations in microbiome and effect of interventions designed to impact microbial community and function during disease and therapy.



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Modelling Host-Microbiome interactions *in vitro*

Many links of microbiota to cancer remain only correlative due to lack of modeling host-microbiome connections. This underlies the critical and urgent need for sophisticated *in vitro* model systems to address the issue, which will help not only to gain mechanistic insights into microbial effects on intestinal epithelium but also will lead to development of innovative therapeutic strategies targeting microbial-epithelial interactions. To overcome this limitation, we used tissue engineering, which is a promising field that aims at developing bioartificial substitutes capable of repairing and regenerating. Organoid technology has significantly improved our understanding of disease-associated multicellular complexity at tissue level. Therefore, by using bioprinting or scaffolds for tissue engineering and 3D organoids, our studies elucidate host and microbial interactions *in vitro*.

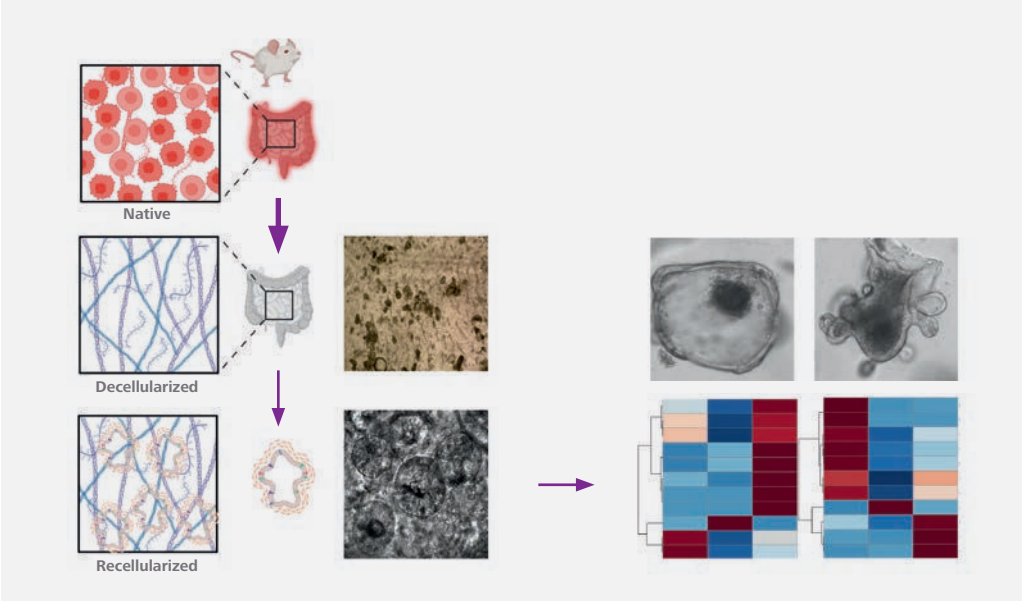

Figure 4.
Mechanistic insights into host-microbiome interactions.

Gewebsinteraktionen und Signalmechanismen im Darmkrebs

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Signaling crosstalk in the colon cancer microenvironment

3D organoid biobanks from human colorectal cancer

Paracrine signaling mechanisms of the intestinal stem cell niche

Targeting of the colon cancer microenvironment

In Germany, colorectal cancer (CRC) is the third most common cancer type with 59,000 new diagnoses and 24,000 death cases each year. Two major classes can be distinguished: microsatellite stable (MSS, ~85%) and microsatellite instable tumors (MSI; ~15%). Great advances have been achieved in tumor prevention and immune checkpoint therapy has shown impressive efficacy in patients with MSI tumors. However, the therapeutic options for patients with MSS tumors are still limited. Main challenges are the high genetic heterogeneity of CRC both at the inter-individual and intratumoral level. In addition, prognosis and therapy responses are strongly influenced by the tumor microenvironment (TME). To better understand the complex link between CRC genotype and phenotype and to develop rational therapies new experimental models are required.

Patient-derived tumor organoids (PDTOs) have emerged as an important preclinical tool. The organoid technology is based on expansion of primary epithelial cells in 3D Matrigel and defined growth factors. Originally developed for the mouse small

Unsere Arbeitsgruppe erforscht die zellulären und molekularen Vorgänge bei der Entstehung von Darmkrebs. Insbesondere interessiert uns die Kommunikation verschiedener Zelltypen in der unmittelbaren Umgebung des Tumors, dem so genannten „Tumormicroenvironment". Dabei nutzen wir „Organoide", ein neuartiges dreidimensionales Gewebekultur-System. Organoide können unter definierten Kulturbedingungen aus humanen Darm-Stammzellen etabliert werden und bilden Darmepithel-spezifische Strukturen wie Krypten (Furchen) oder Villi (Zotten) aus. Dadurch können Stammzellen in einem Gewebe-ähnlichen Zustand expandiert werden, was die Untersuchung von molekularen Signalen in einer definierten Mikroumgebung ermöglicht. Durch Zugabe von Fibroblasten, Gefäß- oder Immunzellen wird der Organkontext nachgebildet. Im Mittelpunkt unserer Forschung steht die genetische Analyse der Entstehung und Progression des Darm-Karzinoms. Im Rahmen einer klinischen Kollaboration am „Frankfurt Cancer Institut", werden dazu „lebende Biobanken" von Patienten-ableiteten Tumor-Organoidlinien angelegt. Mit Hilfe von genetischen Techniken (CRISPR/Cas9) und Hochdurchsatzanalysen wie Genom-/RNA-Sequenzierung und Proteomanalyse, versuchen wir zu verstehen, wie onkogene Mutationen den Tumor-Phänotyp beeinflussen. Im Rahmen des EU-Projekts „EUbOPEN" nutzen wir Organoid-Modelle zur pharmakologischen Testung als Ansatzpunkt für zukünftige Therapien beim Darmkrebs.

intestine, the culture conditions have been adapted to support growth of normal and tumor cells from human colon and other organs. PDTOs can be expanded and cryopreserved to establish 'living biobanks' that represent the tumor heterogeneity among and within patients. In clinical collaboration and supported by Frankfurt Cancer Institute, we are generating a CRC organoid biobank as a research tool to study individual cancer phenotypes that affect drug sensitivity and therapy resistance. In addition, our group develops protocols for genetic modification of 3D organoids.

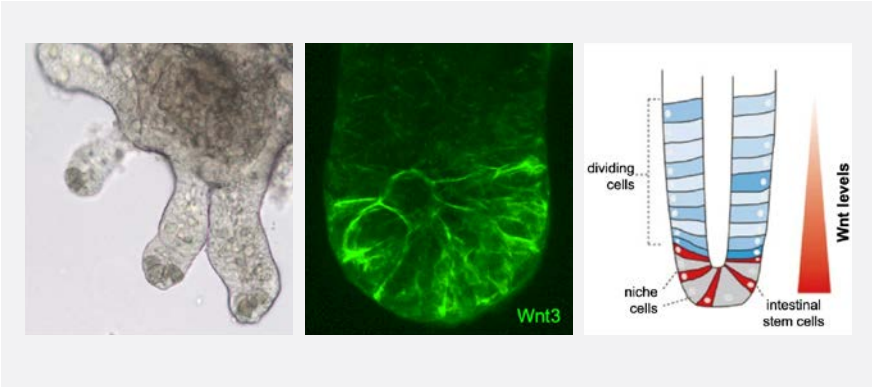


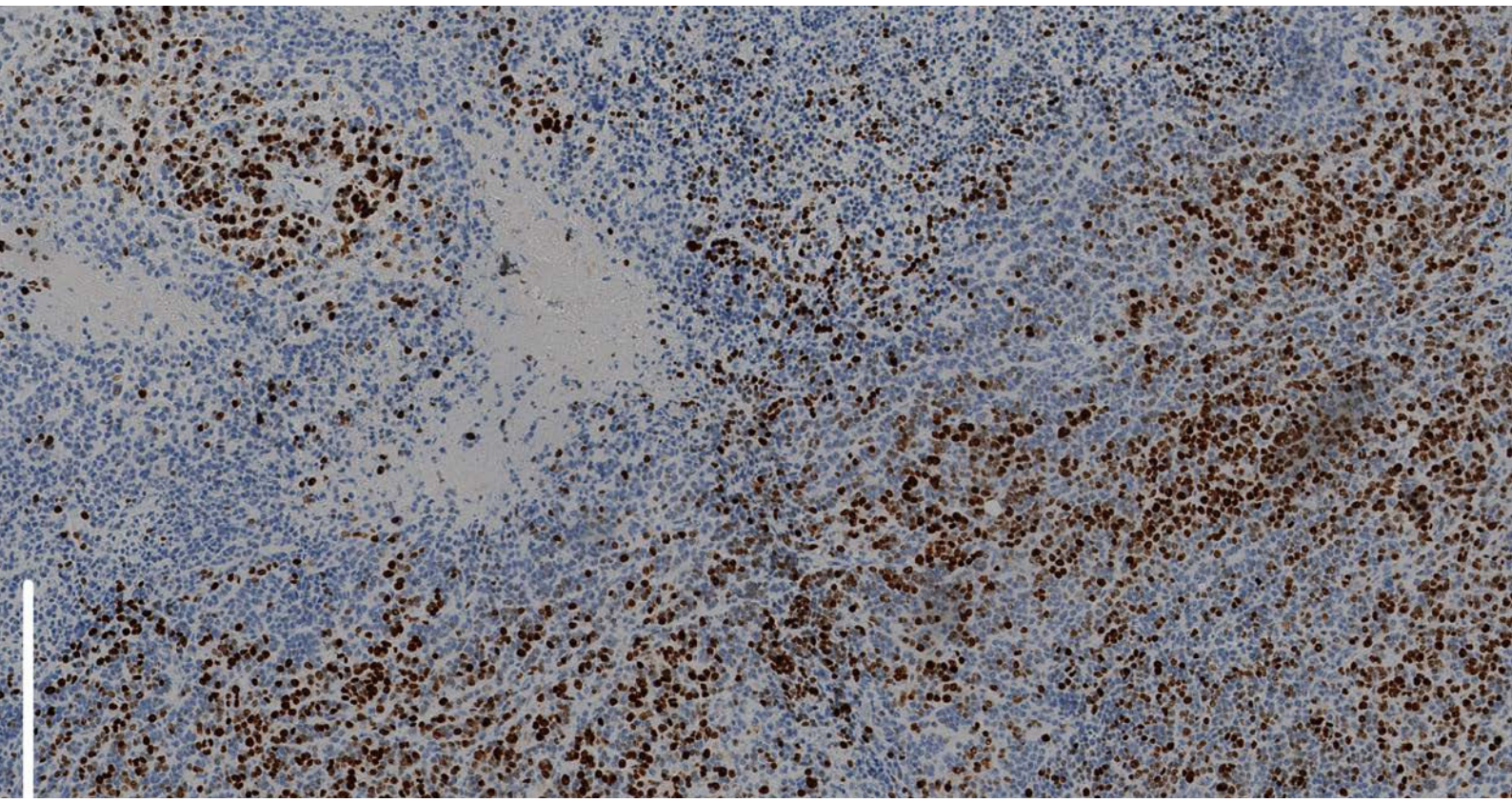
Figure 1.
Study of Wnt signals in self-renewal and differentiation. Left: mouse intestinal organoids. Middle: localized production of Wnt3 by the stem cell niche (from Farin et al., *Nature* 2016). Right: Epithelial patterning by the Wnt3 gradient (adapted from commentary by Gregorieff and Wrana, *Cell Research* 2016).

Main research focus areas are:

I. Wnt signals in stem cell homeostasis and colon tumorigenesis

Tissue homeostasis and regeneration depend on the capacity of stem cells to proliferate and produce differentiated offspring. In the past years, it has been recognized that signals from the surrounding 'stem cell niche' govern epithelial turnover to meet the physiological demands. Small intestinal organoids contain functional stem cells that continuously generate differentiated cells *in vitro*. Using genetic and microscopic techniques, we have identified that the Wnt3 protein is secreted by niche cells, thereby inducing stem cells in close vicinity (Fig. 1; Farin et al., Nature 2016). Localized production and limited mobility of the Wnt3 protein results in the stereotypic arrangement of the epithelium as a mechanism of self-organization.

In addition, we have recently identified that Wnt signaling also affects other cell types in the tumor microenvironment: Using organoid transplantation models, we have shown that cancer-associated fibroblasts (CAFs) are critically dependent on the Wnt pathway. A switch of CAF subtypes in response to Wnt inhibition influenced tumor growth and invasiveness (Mosa et al., Cancer Research 2020), highlighting how stromal cell plasticity can regulate tumor malignancy.



II. Functional genetic screening to identify CRC driver mutations

Oncogenes and tumor suppressors show context-specificity that depends on the tumor type, the genetic background, and environmental factors. We aim to recapitulate this complexity using 3D organoid models. By genetic engineering of patient-derived organoids using the CRISPR/Cas9 technology, we have recently studied the transcriptomic and proteomic changes induced by known oncogenic mutations (Michels et al., *J. Exp. Med.* 2019). To facilitate high-throughput testing of many genes in parallel, we have recently developed a protocol for pooled CRISPR/Cas9 library screening in human colon organoids (Fig. 2; Michels et al., *Cell Stem Cell* 2020). This technology permits unbiased detection of genes that confer positive or negative growth advantages. We have used custom-generated gRNA libraries to identify tumor suppressors *in vitro* and after organoid xenotransplantation. This powerful platform for phenotypic characterization may in future allow to identify patient-specific tumor vulnerabilities.

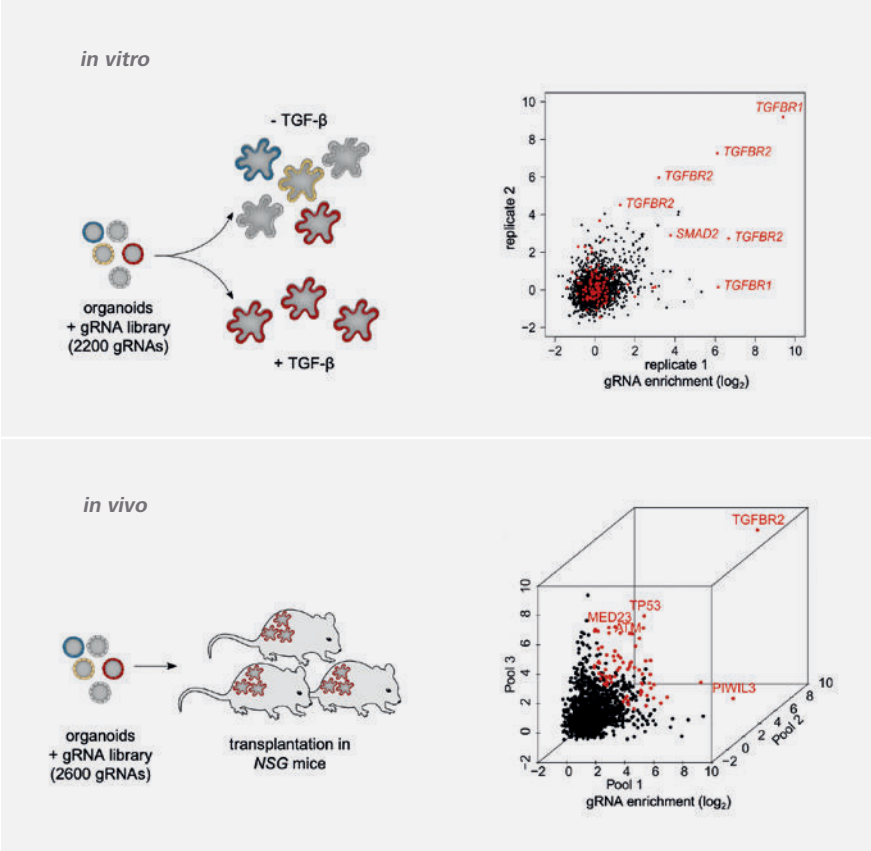
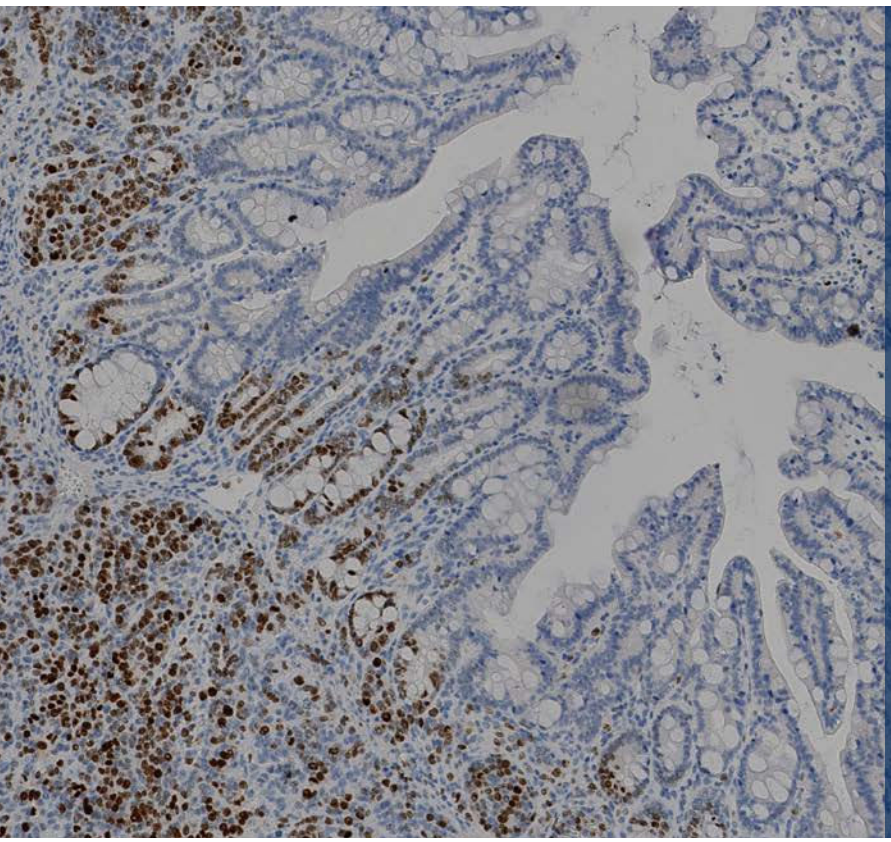


Figure 2.
CRISPR/Cas9 library screening in 3D organoids *in vitro* and *in vivo*
Top: TGF-β resistance screen *in vitro*. Barcode sequencing after phenotypic selection (growth in presence of TGF-β). Bottom: Tumor suppressor screen in human organoids after subcutaneous xenotransplantation. Barcode sequencing in 3 tumor pools. (data from Michels et al., *Cell Stem Cell* 2020).



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*co-correspondence

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3D model for CAR-mediated cytotoxicity using patient-derived colorectal cancer organoids. ***EMBO Journal****; doi: 10.15252/embj.2018100928*

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III. Preclinical organoid models for cancer immunotherapy

In CRC, cell-based immunotherapies could be beneficial, because immune checkpoint inhibitors alone are not effective in the majority of MSS patients. Lymphocytes can be engineered to recognize tumor-associated antigens; however, the application of such chimeric antigen receptors (CAR)-modified cells has proven challenging in solid tumors. The immunosuppressive tumor stroma in CRC prevents effector cell recruitment and function and we furthermore lack predictive *in vitro* models. To address these challenges, we have recently developed a CAR-PDTO co-culture system (Fig. 3; Schnalzger et al., *EMBO Journal* 2019). In collaboration with Prof. Winfried Wels (Georg-Speyer-Haus), cytotoxic killing by CAR-modified NK-92 cells was measured in an enzymatic assay and by live imaging, providing a preclinical platform to evaluate efficacy and specificity CAR therapies.

As participant of the EU-consortium 'EUbOPEN' ('Enabling and unlocking biology in the OPEN' 2020-2024), we have developed a PDTO drug screening platform. The EUbOPEN consortium is funded by the Innovative Medicines Initiative (IMI2) and aims to generate an open access chemogenomic library of compounds covering the 'druggable human genome'. Together with our partners from academia and pharmacologic industry, we develop 'Human Tissue Assays' for CRC. We conduct high-throughput pharmacologic screens using our organoid biobank models to identify new therapeutic strategies.

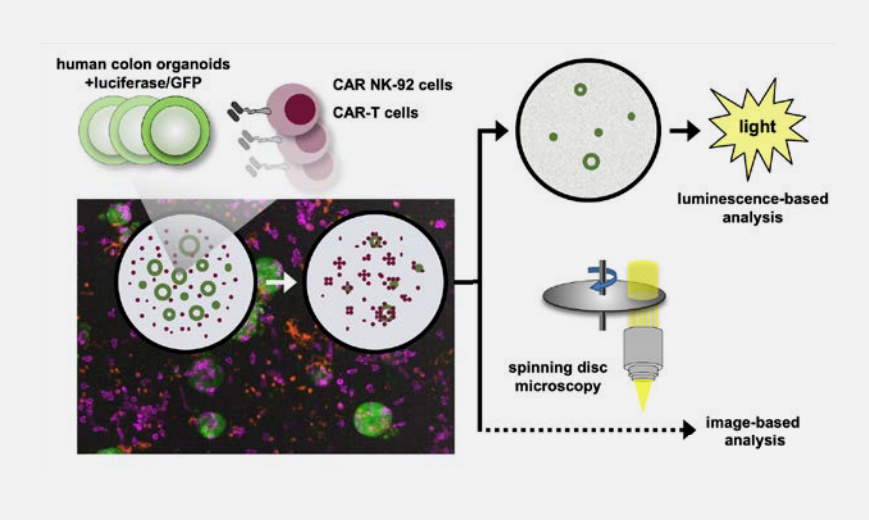

Figure 3.
3D model for CAR-mediated cytotoxicity using patient-derived CRC organoids
Combination of GFP/luciferase transgenic human colon cancer organoids (green) with CAR-cells (violet). Monitoring of cytotoxic responses by video microscopy and enzymatic read-outs (adapted from Schnalzger et al., *EMBO Journal* 2019).

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Modulation des Tumormikromilieus als Chance für die Therapie

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Inflammatory pathways in intestinal carcinogenesis

Tumor microenvironment

Colorectal carcinoma

Modulation of immune responses, intracellular pathways and inflammatory responses

Colorectal carcinoma (CRC) is one of the three most frequent types of cancer in industrial nations. Despite improvements in preventive colorectal cancer screening and treatments, colorectal cancer remains one of the top three causes of cancer-related deaths. Genetic predisposition as well as age increases the risk of attaining malignantly transformed intestinal epithelial cells (IEC), simply by an accumulation of mutations over time. Next to these immutable reasons, life style factors like "western" diet, obesity or smoking influence tumor progression, forming an underlying inflammatory milieu. Inflammation is involved in all stages of cancerous disease, from initiation (DNA damage and epigenetic changes), to tumor-elicited and even therapy-induced inflammation [Schmitt and Greten, 2021, Denk and Greten, 2022]. Notably, inflammation in cancer is a double-edged sword, welcomed in accelerating anti-tumor immune responses but undesirable due to promotion of angiogenesis, proliferation and metastasis. Its ability to alter cell polarization majorly contributes to the plasticity of CRC and adds to the enormous complexity of the network of tumor initiating cells, stroma (endothelial cells, cancer associated fibroblasts (CAFs), invading immune cells), the components of the microbiota and soluble mediators of all those involved. With all these different parameters that can be individually influenced, the heterogeneity between tumors of different patients and even in-between the same patient or tumor is not surprising.
In the last decades the tumor microenvironment (TME) came into focus of research and thus a target for therapy. Modulating antitumor T cell responses by intervening with T cell exhaustion by checkpoint inhibitors was a breakthrough for different cancers. Unfortunately, these checkpoint inhibitors act only in a small fraction of CRC patients that exhibit microsatellite instability. Improvement of therapies and detection of new targets is therefore of ongoing importance. By studying the components of the TME and its interactions and processes in mouse models and human samples, we have recently been able to identify several novel therapeutic pathways, some of which will be evaluated in clinical trials. In the course of her doctoral thesis and close cooperation with the department

Der Fokus unserer Forschung liegt auf der funktionellen Analyse des Mikromilieus im Kolonkarzinom und der Nutzung aufgedeckter Prozesse zur therapeutischen Intervention. Hierbei kommen moderne dreidimensionale *in vitro* Kulturen muriner und humaner intestinaler Epithelzellen, sowie relevante Mausmodelle zum Einsatz, welche die verschiedenen Arten und Stadien der kolorektalen Karzinogenese valide abbilden.

Durch die systematische Analyse des Tumormikromileus, seiner Vielzahl von Zellen, löslicher Mediatoren und der ablaufenden intrazellulären Signalwege, konnten wir verschiedene Zusammenhänge aufdecken, die in diesem Jahr zur Initialisierung zweier klinischer Studien führte.

Eine dieser Studien soll zur Verbesserung der Radiotherapie beitragen. Wir konnten feststellen, dass auftretende Resistenzen zum Teil damit begründet werden können, dass Interleukin-1α-induzierte, inflammatorische Tumor-assoziierte Fibroblasten eine Seneszenz unterlaufen, die zur Ausschüttung von Wachstumsfaktoren und Komponenten der extrazelullären Matrix führt. Dadurch wird ein tumorförderndes Mikromilieu erzeugt, welches den Effekt der Bestrahlung kompensieren kann. Durch die Blockierung des IL-1-Signalweges in Kombination mit der Radiotherapie soll dieser Prozess verhindert werden. Ebenso konnten wir neue Erkenntnisse über die Resistenz gegenüber Chemotherapeutika gewinnen. Auch in diesem Fall wird ein kompensierender Resistenzmechanismus durch umliegende Zellen vermittelt, der mithilfe einer Kombinationstherapie unterbunden werden kann. Des Weiteren konnten wir eine vielversprechende Substanz identifizieren, die zur verbesserten anti-Tumor T-Zellantwort beiträgt und deren Effekt auf den Menschen ebenfalls durch eine klinische Studie genauer untersucht werden soll.

All diese vielversprechenden neuartigen Therapieansätze basieren auf unserer jahrelangen Forschung zugrundeliegender molekularer Prozesse und zellulärer Interaktionen des Tumormikromilieus und zeigen die großen Vorteile, dieses neben den Tumorzell-spezifischen Standard-Therapien nutzbar zu machen.

of radiology of the University Hospital Frankfurt, Adele Nicolas identified IL-1α as a druggable component in rectal cancer patients receiving neoadjuvant therapy [Nicolas et al., 2022]. Analyzes of clinical data revealed a strong enrichment of IL-1/TNFα-dependent inflammatory CAFs (iCAFs) in tumor biopsies of radioresistant, non-responding patients. To uncover the underlying mechanisms, a mouse model was established in which genetically modified colorectal organoids were orthotopically transplanted and subjected to fractionated irradiation [Fig. 1A].

Organoids contained CRC-typical mutations in ***A**pc*, *Tr**p**53*, ***T**gfbr2*, and ***K**-ras* (APTK) and / or additional myristoylated **A**KT (APTKA). Interestingly, while APTK tumors responded well to radiotherapy, APTKA-derived tumors not only showed resistance but increased invasion and metastasis following radiation *in vivo*, although both of them responded equally well *in vitro*. It could be shown, that conditioned media from APTKA but not APTK organoids polarized naïve fibroblasts to an inflammatory phenotype, similar to iCAFs and that this effect was mediated by IL-1α. To examine whether tumor derived IL-1α causes the observed radio resistance in APTKA tumors *in vivo*, mice received combination therapy of radiation and the IL-1 receptor antagonist anakinra. Indeed, the combinatorial treatment abrogated tumor growth and invasion, that could not be observed by either single treatment alone [Fig. 1B]. To underline the significance of CAFs in this context, genetically modified mice were used, where the gene for the IL-1 receptor Il1r1 could be deleted specifically in fibroblasts (*Col1a2CreER*^{T2}/*Il1r1*$^{flox/flox}$ mice). Remarkably, a reduction in tumor size and invasion upon radiation was also observed in this model, where

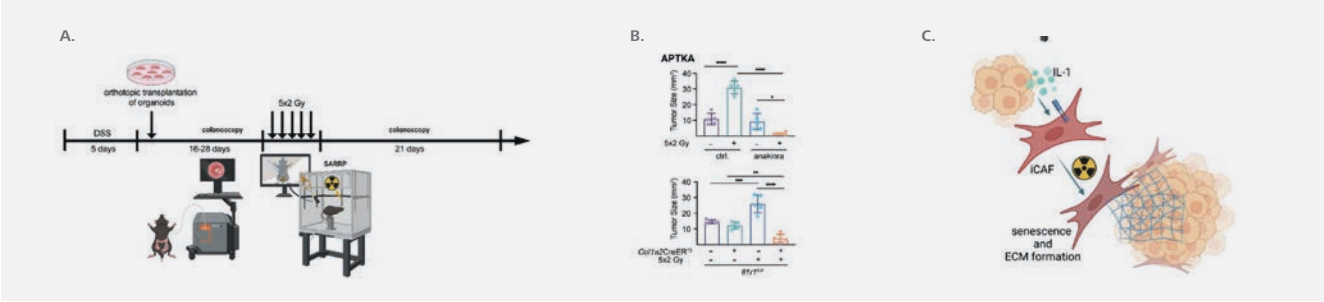
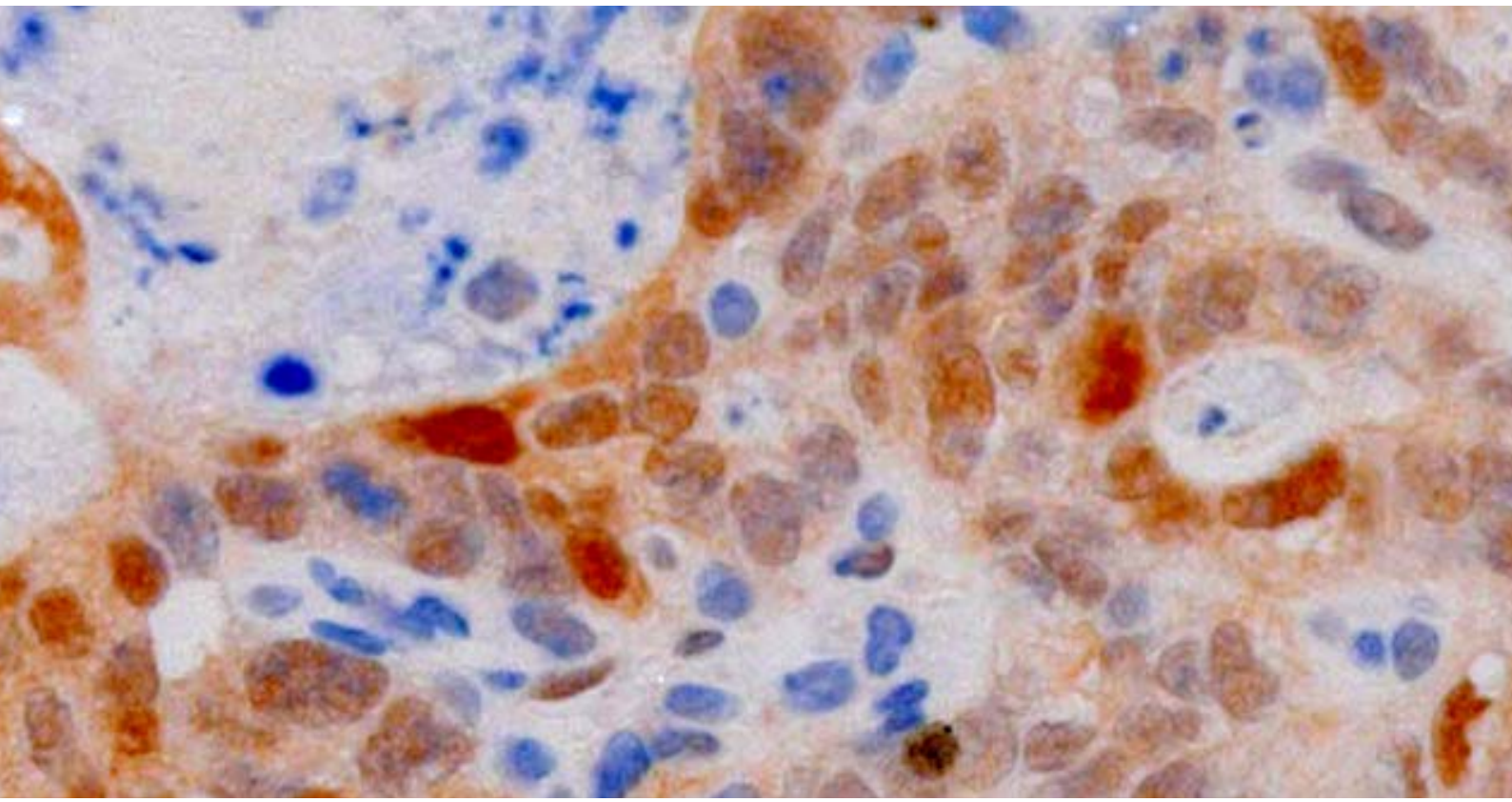


Figure 1.
IL-1 dependent modulation of radiotherapy resistance. (A) Preclinical colorectal carcinoma model. After inducing colitis by dextran sodium sulfate (DSS) in drinking water, organoids are injected intrarectally into mice. Tumors are observed by colonoscopy and locally irradiated (5x 2 Gy) using the small animal radiation research platform (SARRP). (B) Example of orthotopic APTKA tumors with fractionated irradiation and/or anakinra treatment in wildtype mice (upper panel) or conditional IL-1R ablation in fibroblasts (*Col1a2CreER*^{T2}/*Il1r1*^{flox} mice, lower panel). (C) IL-1α polarizes CAFs into iCAFs, that react to radiotherapy with senescence and the formation of extracellular matrix (ECM), promoting tumor growth and counteracting therapy-induced death of tumor cells.



IL1-signaling was specifically prevented solely in CAFs [Fig. 1B, lower panel]. A series of elegant experiments clarified that in IL-1-polarized iCAFs, irradiation induces p53-dependent senescence associated with severe DNA damage and an increase of extracellular matrix (ECM) components and growth factors. The secretion of those ECM constituents and cytokines support tumor growth, invasion and metastasis, counteracting the radiotherapy-induced tumor cell death [Fig. 1C]. These findings could be confirmed in the human setting, where IL-1 receptor antagonist (IL-1RA) serum levels were found to be reduced in radio-resistant non-responders. Together, these valuable insights in IL-1- and iCAF-dependent radio resistance mechanisms paved the way for the initiation of a clinical trial using anakinra as a combinatorial treatment to standard of care radiation therapy.

This work illustrates very well, how certain cells can affect their adjacent cells, altering therapy responses. A similar occurrence could be shown in another therapeutic setting. To determine resistance mechanisms in CRC patients after chemotherapy, we treated patient derived tumor organoids (PDTO) with the standard of care chemotherapeutic 5-Fluorouracil (5-FU) *in vitro* and analyzed surviving organoids. Enhanced phosphorylation of two kinases involved in mTORC1 (mammalian target of rapamycin complex 1) regulation could be observed. To investigate if mTOR activation is involved in chemotherapy resistance mechanisms, PDTOs were treated with the mTOR inhibitor rapamycin in combination with 5-FU. Indeed, the combined compounds suppressed PDTO reseeding in vitro as well as growth of subcutaneously transplanted PDTOs *in vivo* [Schmitt et al., *Nature* in press]. To unravel the underlying mechanism, a mouse model was used, that allows to study the consequences of cell death by targeting just a subset of tumor cells without applying a chemotherapy that affects all tumor cells. Specific depletion of Lgr5 expressing cells in colorectal tumors grown in *Lgr5*$^{EGFP\text{-}DTR}$ mice by administration of diphteria toxin (DT) resulted in the phosphorylation of mTOR in neighboring LGR5 negative cells. Again, double treatment of DT and rapamycin could diminish organoid reseeding *in vitro* and caused regression of subcutaneous tumors *in vivo*, which could not be achieved by either treatment alone. Further mechanistic analyzes revealed that the observed mTOR dependency in LGR5 negative tumor cells was caused by severe DNA damage from reactive oxygen species (ROS) derived from apoptotic LGR5 positive target cells, that was compensated by mTOR upregulation via ATP, released by the dying cells as well [Fig. 2]. While killing LGR5+ cells only caused tumor stasis, the additional administration of rapamycin induced apoptosis in ROS-affected neighboring LGR5- cells and caused tumor shrinkage. Similarly, inhibition of P2X4 the receptor

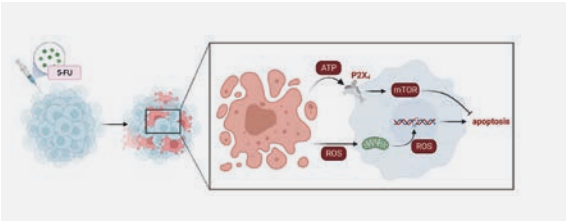
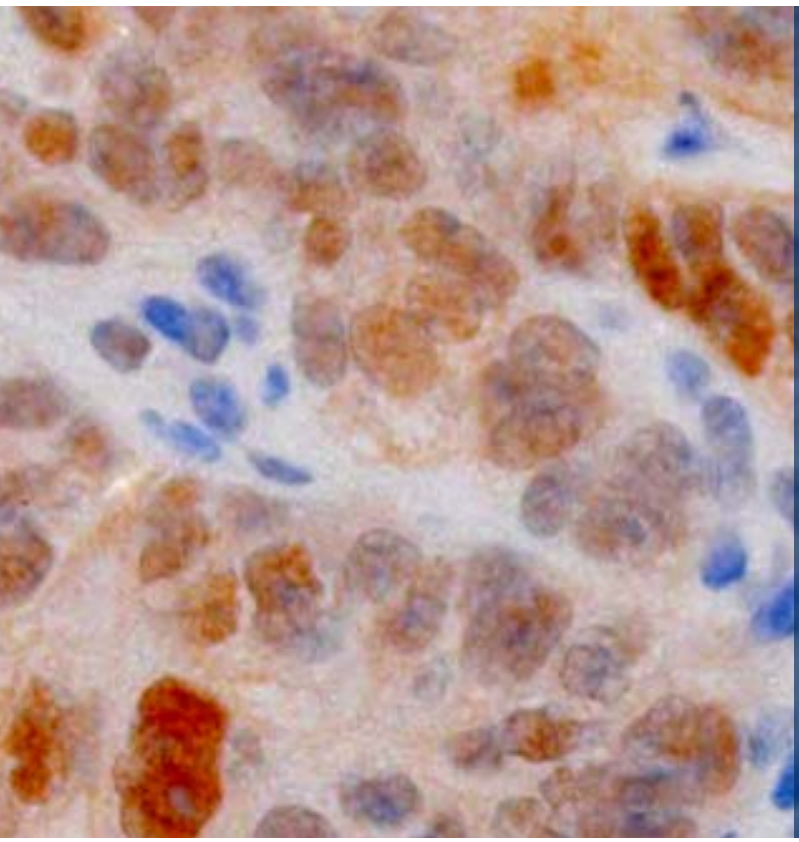


Figure 2.
Chemotherapy induced cell death leads to ROS-dependent apoptosis induction in neighboring cells. At the same dying cells release ATP that triggers mTOR via P2x4. Inhibition of P2x4 or mTOR in combination with standard chemotherapy causes rapid tumor regression.



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Inflammatory fibroblasts mediate resistance to neoadjuvant therapy in rectal cancer. ***Cancer Cell.*** *2022 Feb 14;40(2):168-184.e13.*

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responsible for ATP-dependent mTOR activation led to massive tumor regression when 5-FU was applied suggesting a new mode of combination therapy.

Immune checkpoint inhibition represents a relevant therapy option for microsatellite-instable colorectal tumors, which however comprise only small subset of CRC. Thus, finding ways to render microsatellite-stable tumor sensitive to this approach are urgently needed. Following up previous findings, linking mitophagy in intestinal epithelial cells to increased MHC-I mediated antigen presentation and consequent CD8-dependent anti-tumor response [Ziegler et al., 2018], we aimed to assess the impact of pharmacologically induced mitophagy for CRC treatment. Urolithin A (UA), which can be metabolized by intestinal bacteria from components of pomegranate, but is also available as a dietary supplement, is a potent inducer of mitophagy. Mice that were fed with UA-containing diet showed significantly less chemically induced colon adenomas and reduced growth of subcutaneously transplanted APTK tumors accompanied by increased numbers of tumor infiltrating T cells. Interestingly, the tumor suppressive effect of UA was strictly dependent on the presence of T cells. We were able to demonstrate that UA triggered the formation T memory stem cells (T_{SCM}) upon UA treatment of isolated naïve T cells in vitro as well as in APTK tumors *in vivo*. This subset is characterized by extreme longevity and self-renewal, providing fresh effector T cell progeny over a prolonged period of time. Indeed, adoptively transferred, antigen specific OT-I CD8 T cells showed better engraftment and APTK-OVA tumor suppression upon UA pretreatment. Similar results could be obtained by UA treatment of human engineered chimeric antigen receptor (CAR) T cells. When UA was present during the generation of CAR T cells, the number of TSCM were markedly increased *in vitro*, thus improving CAR T cell fitness and efficacy. We were able to determine the underlying mechanism for the UA-induced T cell phenotype which was associated with the release of the mitochondrial phosphatase PGAM5 (phosphoglycerate mutase family member 5) into the cytoplasm, where it blocks β-catenin degradation leading to subsequent activation of WNT target genes including *TCF7*, a prerequsiste for T_{SCM} formation [Fig. 3]. These results will be now evaluated in a phase I study to assess whether a similar phenotype can be induced in healthy individuals upon oral UA administration, which will serve as the basis for further studies in cancer patients receiving immunotherapies.

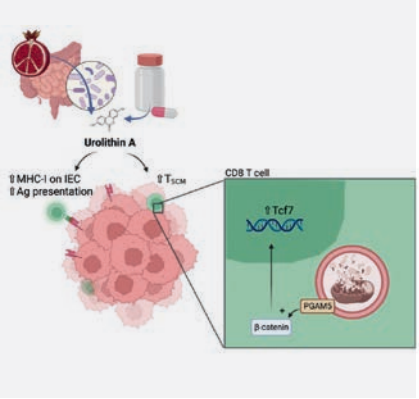

Figure 3.
Urolithin A mode of action. Urolithin A is a metabolic product from pomegranates that shows dual anti-tumorigenic properties. It enhances MHC-I expression and subsequent antigen presentation by IECs. On the other hand, mitophagy in CD8 T cells causes release of PGAM5 by degraded mitochondria, which prevents β-catenin degradation and subsequent activation of WNT target genes.

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Die Rolle der Tumormikroumgebung in der Hirnmetastasierung

Microenvironmental regulation of brain metastasis

CNS immune landscape

Brain metastasis-associated inflammation

Metabolic checkpoints

Resistance mechanism

Brain metastasis remains an unmet clinical need in high demand for novel therapeutic options to overcome tissue-specific limitation of treatment success. Detailed knowledge on cellular and molecular drivers of disease progression and therapy response is required to provide scientific rationale for the development of innovative combination therapies. We therefore seek to gain insight into the complex interplay of tumor-associated stromal or immune cells with tumor cells at distinct stages of the metastatic cascade.

Adaptive resistance mechanism blunts long-term efficacy of immune-targeted therapies

The development of targeted- or immunotherapies has revolutionized intervention strategies for different primary cancers. However, response rates vary among distinct tumor types and individual patients. Moreover, metastases often show lower response rates compared to primary tumors. The microenvironment represents a critical factor that determines disease progression and the outcome of therapeutic intervention. Given the immune-privileged status of the central

Die Einführung von zielgerichteten- oder Immuntherapien in der Klinik hat große Fortschritte in den Behandlungsmöglichkeiten vieler Krebserkrankungen erzielt. Metastasen stellen jedoch weiterhin die Haupttodesursache bei Tumorpatienten dar, da die verfügbaren Behandlungsmöglichkeiten, insbesondere bei Hirnmetastasen, nur begrenzt wirksam sind. Bei der Entwicklung neuartiger Therapieansätze zur Bekämpfung von Hirnmetastasen ist es daher wichtig, gewebsspezifische Hürden, die zu Therapieresistenzen führen, zu verstehen und diese gezielt zu überwinden.

Das Forschungsziel unserer Nachwuchsgruppe besteht darin, die komplexen Interaktionen zwischen Tumorzellen unterschiedlicher Entitäten (Melanom, Bronchial- oder Mammakarzinom) und hirnresidenten- sowie rekrutierten Zelltypen während der Hirnmetastasierung zu entschlüsseln und Therapieansätze zu entwickeln. Ein besonderer Fokus liegt hierbei auf der Aufklärung von Resistenzmechanismen, die eine dauerhafte anti-tumor Immunantwort verhindern.

nervous system (CNS), brain metastases (BrM) represent a particularly challenging entity for successful immunotherapy. Even though BrM induce the recruitment of myeloid and lymphoid cells into the CNS, the environment poses an immune suppressive pressure to prevent tissue-damaging inflammation. We previously employed different strategies to target either tumor-promoting functions of tumor-associated myeloid cells or reactivate anti-cancer functions of tumor-infiltrating T cells.

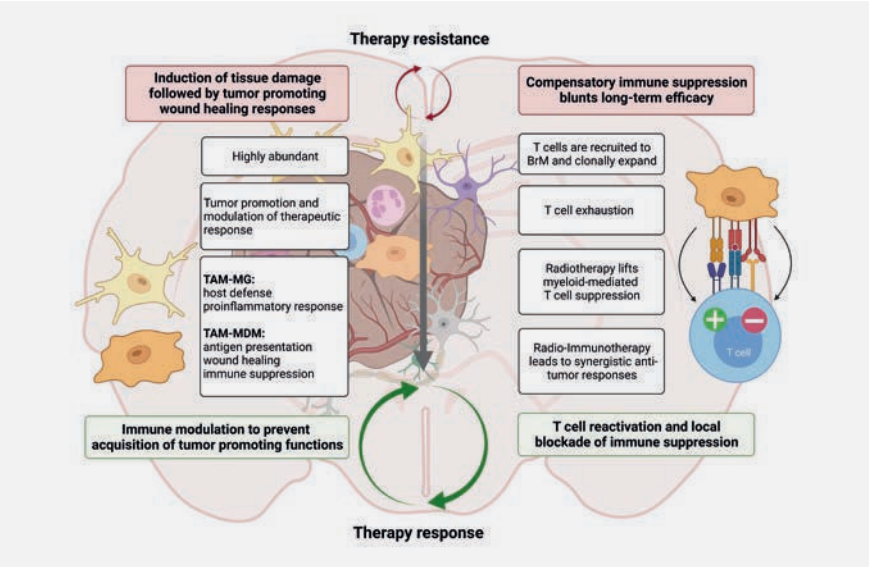
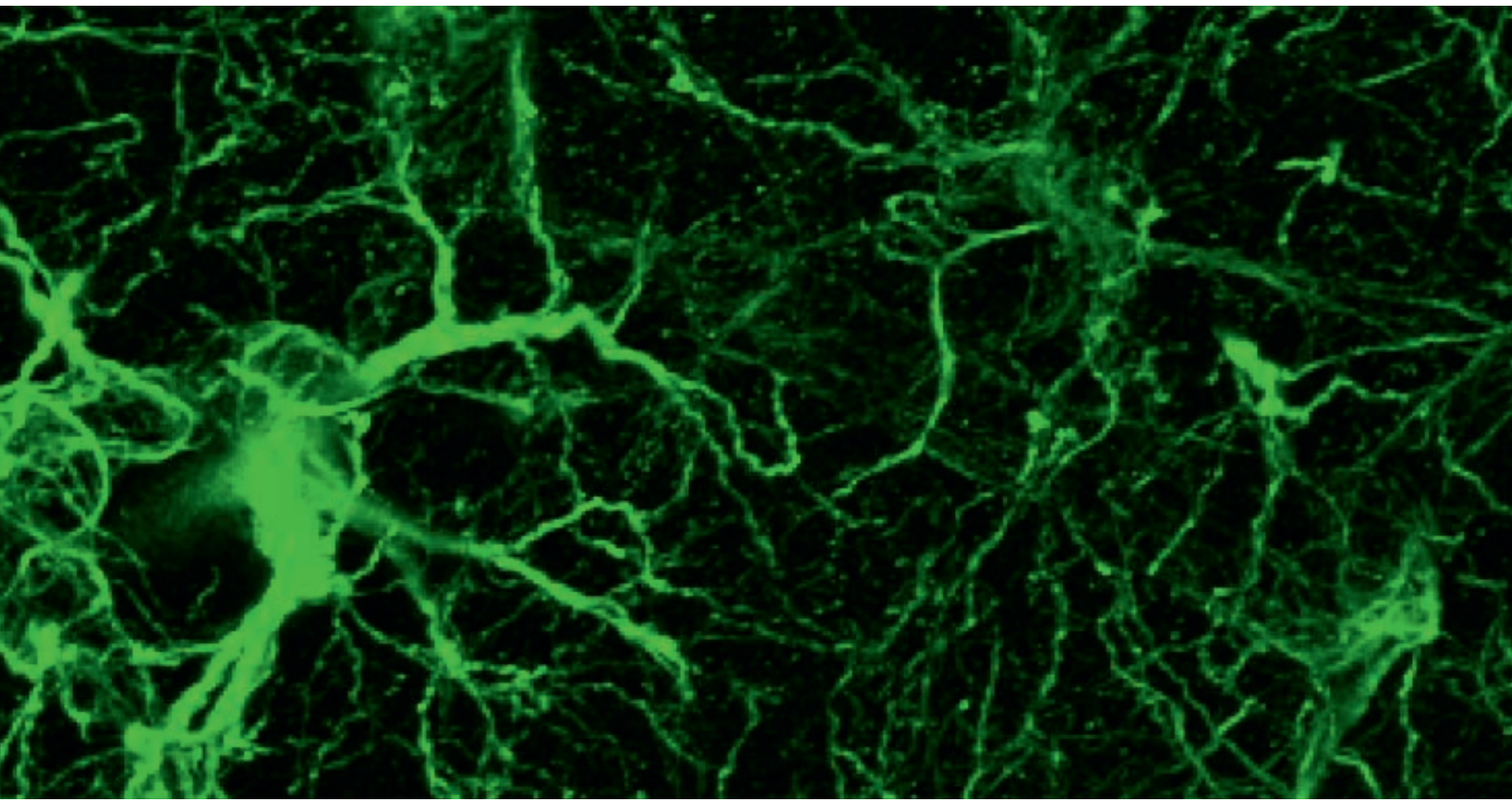


Figure 1.
Model figure summarizing key characteristics of the lymphoid and myeloid compartment in brain metastases. Lymphoid and myeloid-targeted therapies show limited efficacy in brain metastases due to tissue specific limitations to therapeutic efficacy. Combination therapies have to be developed to target tumor promoting functions of cancer-associated immune cells and to maintain or induce anti-cancer immune responses to achieve sustained tumor control with minimal risk of inducing neurotoxicity. Figure was created using Biorender.

Our data revealed prominent anti-cancer effects in response to TAM-targeted therapies using the CSF1R inhibitor BLZ945. However, induction of compensatory CSF2-mediated macrophage activation that led to tissue damaging inflammation blunted longer-lasting anti-tumor efficacy. Likewise, we observed anti-tumor efficacy of radio-immunotherapy resulting in enhanced recruitment of T lymphocytes. However, anti-cancer T cell responses were suppressed by monocyte-derived macrophages that show upregulation of immune checkpoint molecules in response to radio-immunotherapy. Hence, immune-modulatory strategies that shift the immune suppressive milieu into an inflamed environment is expected to allow for more efficient and durable anti-cancer T cell responses and synergy with immune checkpoint blockade. We therefore seek to gain detailed insight into the complex interplay between innate and adaptive immunity in BrM to provide scientific rationale for the development of combination therapies that aim to block immune-suppression while promoting effective anti-tumor responses with minimal risk to induce adaptive resistance mechanism (Fig. 1)



Novel concepts for immune-targeted therapies

Major limitations of previously tested strategies stem from the rapid induction of acquired resistance mechanisms. We therefore seek to develop novel therapeutic avenues that allow for specific targeting of disease-associated phenotypes of tumor-infiltrating immune cells concomitant with a local relief of immune suppression for efficient T cell effector functions. Gene expression analysis revealed a critical role of metabolic checkpoints in modulating activation states and effector functions of tumor-associated myeloid and lymphoid cells. In particular the purine-adenosine axis acts as a switch between immune suppression and

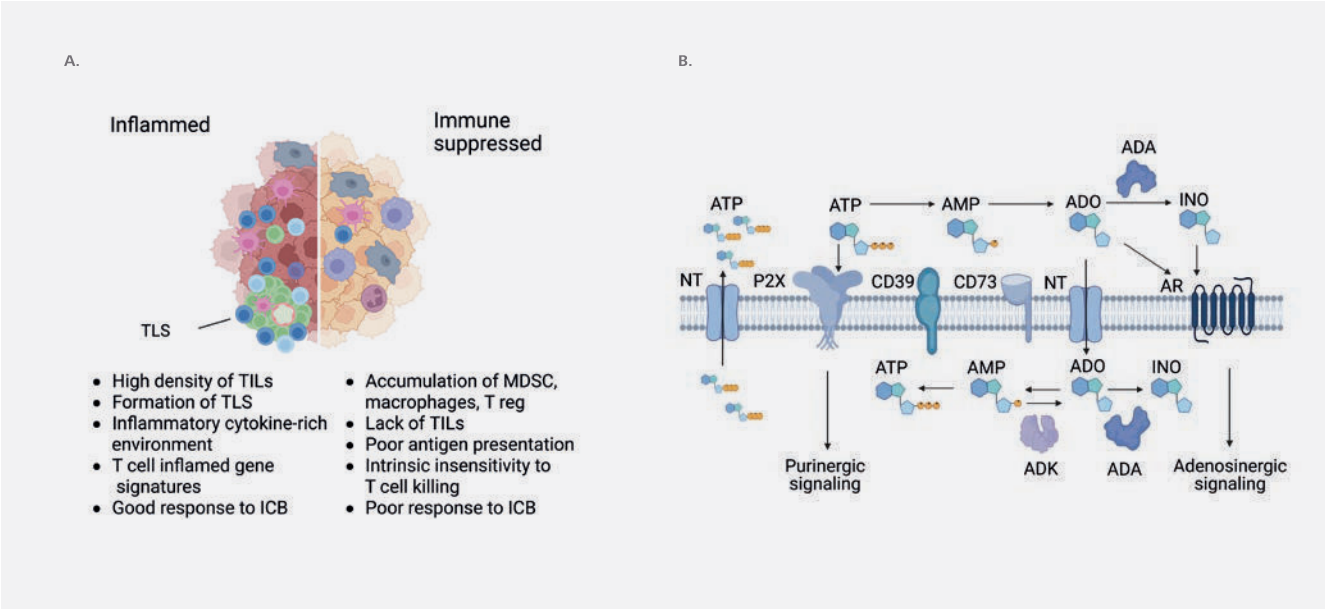
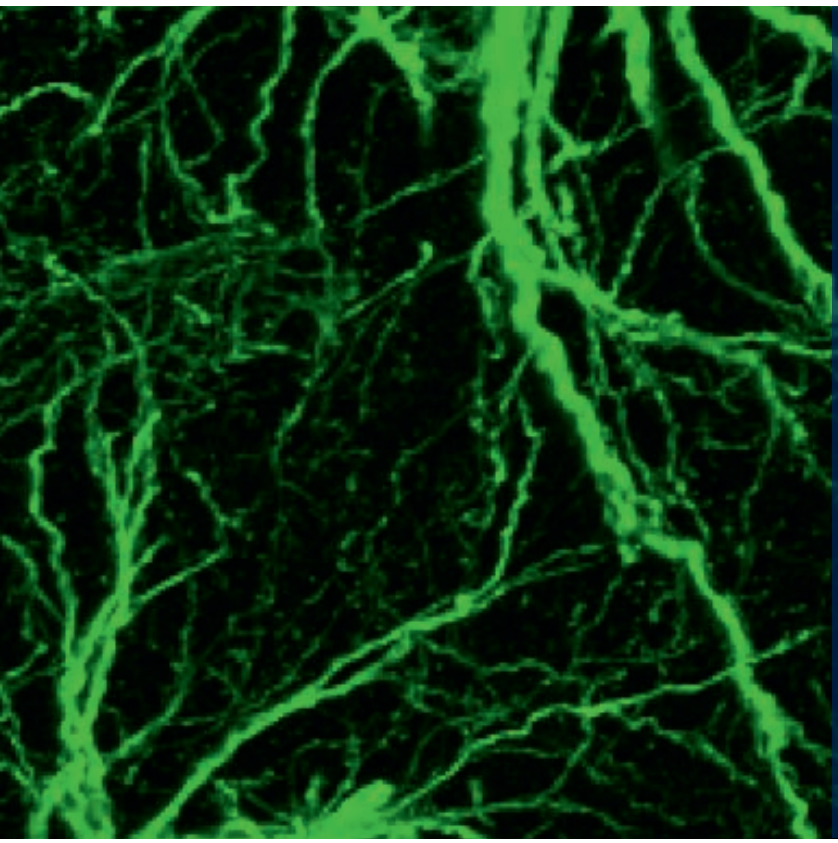


Figure 2.
(a) Tumors can be divided into immunologically hot (=inflamed) and cold (= immune suppressed) environments based different factors such as the ratio of effector cells to suppressive cells and as well as abundance of specific cytokines. (b) The purine-adenosine axis has been identified as an immune modulator in which purinergic signaling drives inflammation, whereas adenosinergic signaling is associated with immune suppression.



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(2020) ***iScience*** *23(6):101178*

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inflammation and expression of different components of the signaling pathway was found in several tumor-associated immune cell types. Genetic and pharmacological inhibition of adenosinergic signaling in combination with radiotherapy leads to significantly improved survival of brain metastasis bearing mice and results in reactivation of exhausted T cells and induction of anti-cancer immunity. Further immunophenotyping will shed light on immune-modulatory effects in different myeloid and lymphoid subpopulations and functionally link the observed phenotypes with cellular functions. In the future, we seek to expand our studies to additional metabolic checkpoints across different tumor entities that metastasize to the brain as well as other metastatic sites such as liver, bone and lung metastasis.

III

Experimentelle Therapie
Experimental Therapy

CAR-exprimierende Lymphozyten für die adoptive Krebs-Immuntherapie

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CAR-engineered lymphocytes for adoptive cancer immunotherapy

natural killer cells

chimeric antigen receptors

bispecific antibodies

Expression of chimeric antigen receptors (CARs) in cytotoxic lymphocytes constitutes a promising strategy for adoptive cancer immunotherapy with effector cells of defined specificity. CARs consist of a tumor-specific single-chain antibody fragment (scFv) connected via a flexible spacer and a transmembrane domain to intracellular signaling domains such as CD3ζ chain or CD3ζ combined with one or more costimulatory protein domains. CAR-engineered T cells have demonstrated remarkable clinical efficacy in patients with malignancies of B-cell origin. Natural killer (NK) cells represent another valuable effector cell population for adoptive cancer immunotherapy, with CAR-NK cells gaining increasing interest. NK cells are part of the innate immune system and play an important role in cancer immune surveillance. NK cells can also modulate T-cell mediated antitumor immune responses by maintaining the quality of dendritic cells and enhancing the presentation of tumor antigens. Nevertheless, in cancer patients NK cells are often functionally compromised due to the immunosuppressive activity of the tumor. Hence, for adoptive cancer

Ziel unserer Arbeiten ist die Erforschung und Entwicklung effektiver Immuntherapien zur Behandlung von Krebserkrankungen. Einen Schwerpunkt bilden dabei natürliche Killerzellen (NK-Zellen), die Teil des angeborenen Immunsystems sind und eine wichtige Rolle bei der Abwehr maligner Zellen spielen. Durch Expression sogenannter chimärer Antigenrezeptoren (CARs) generieren wir genmodifizierte NK-Zellen, die Tumorzellen selektiv abtöten. CARs tragen ein extrazelluläres Antikörperfragment mit Tumorzellspezifität, das über eine flexible Verbindungsregion und eine Transmembrandomäne mit intrazellulären Signaldomänen verbunden ist. Damit lösen die Rezeptoren nach Zielzellerkennung gerichtete zytotoxische Aktivität der Effektorzellen aus. Daneben modulieren CAR-NK-Zellen indirekt auch die endogene adaptive Anti-Tumor-Immunantwort. Als Zielantigene nutzen wir tumorassoziierte Oberflächenantigene wie das zelluläre Proto-Onkogen ErbB2 (HER2), den epidermalen Wachstumsfaktor-Rezeptor EGFR, Liganden des Rezeptors NKG2D und Differenzierungsantigene wie CD19 und CD20. Eine in enger Kooperation mit akademischen Partnern am Standort Frankfurt generierte ErbB2-spezifische Variante der klinisch nutzbaren humanen NK-Zelllinie NK-92 wird gegenwärtig in einer Phase-I-Studie bei Patienten mit rezidiviertem, ErbB2-positivem Glioblastom eingesetzt (CAR2BRAIN; NCT03383978, clinicaltrials.gov).

immunotherapy donor-derived allogeneic NK cells are preferred since they do not recognize tumor cells as 'self', thereby bypassing inhibitory signals.

Tumor-specific natural killer cells

Similar to donor-derived primary NK cells, the continuously expanding human NK cell line NK-92 has been safely applied in clinical trials as an allogeneic cell therapeutic, with durable responses observed in some of the cancer patients treated. In previous work we demonstrated that this therapeutic utility of NK-92 can be further enhanced by expression of CARs which specifically recognize tumor-associated surface antigens expressed by hematologic malignancies or solid tumors. Together with colleagues at the Frankfurt University Hospital we also extended this strategy to primary NK cells and cytokine induced killer cells. In a current approach, we harnessed the broad tumor specificity of the activating receptor Natural Killer Group 2D (NKG2D) in a CAR design. NKG2D has multiple membrane-anchored ligands, which are widely expressed in almost all cancer types. However, shedding or downregulation of such ligands can prevent NKG2D activation, resulting in escape of cancer cells from NKG2D-dependent immune surveillance. To enable tumor-specific targeting of NKG2D-expressing effector cells independent of membrane-anchored NKG2D ligands, we generated a bispecific antibody which can simultaneously bind to NKG2D and the tumor-associated antigen ErbB2 (HER2). On its own, this NKAB-ErbB2 molecule mediated lysis of antigen-positive cancer cells by NK and T cells that naturally express NKG2D. But when it was applied together with NK

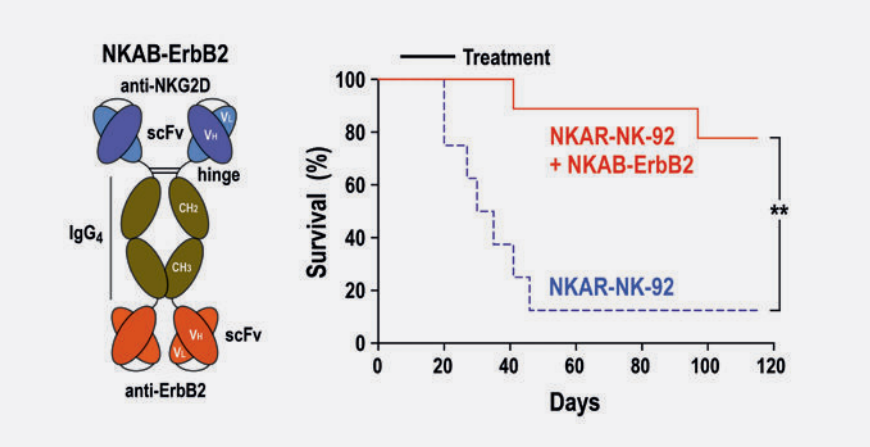
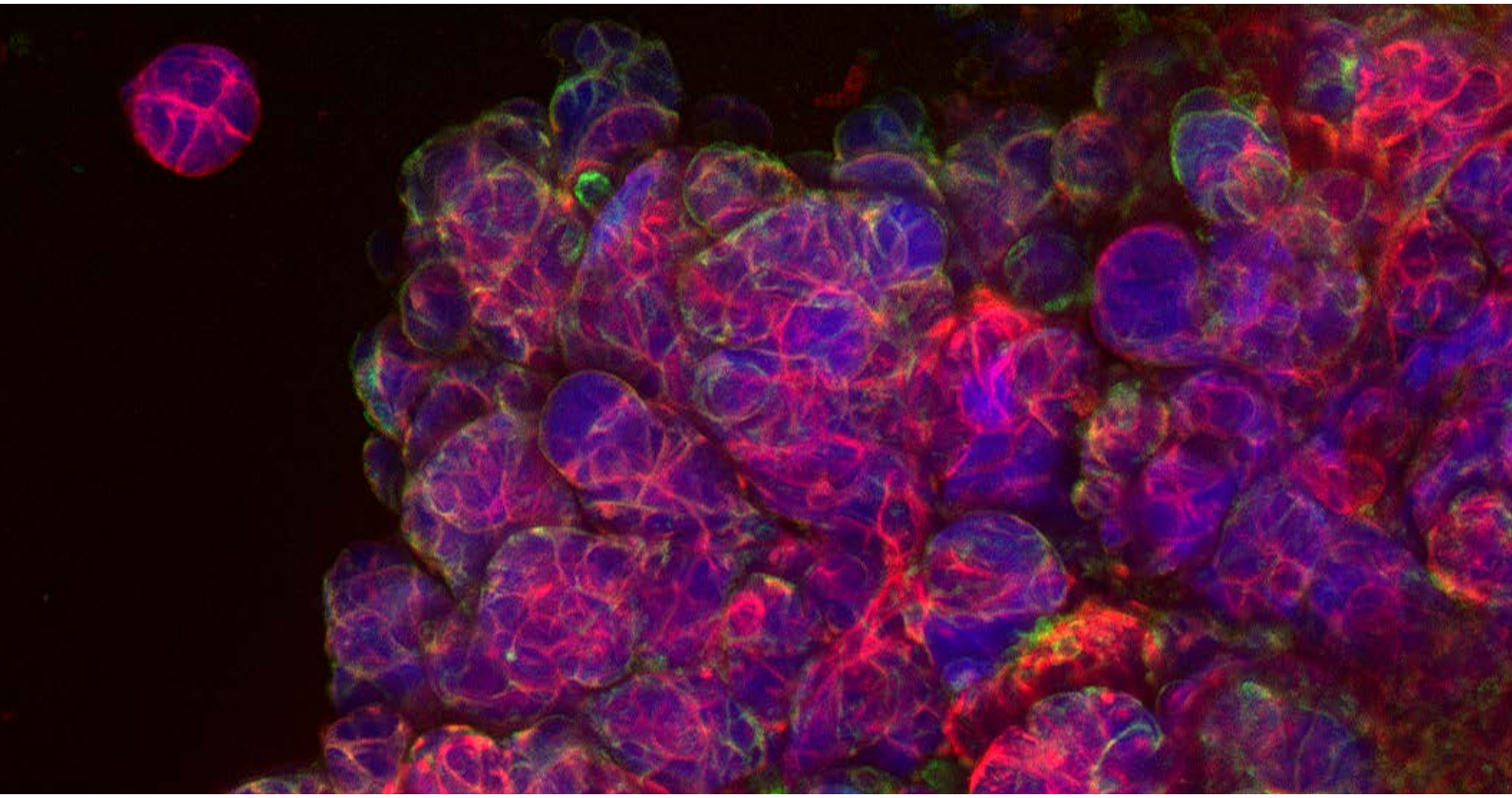


Figure 1.
Bispecific antibody NKAB-ErbB2 redirects NKG2D-CAR expressing NK cells to ErbB2-positive tumors. Left panel: Schematic representation of bispecific antibody NKAB-ErbB2 consisting of an N-terminal NKG2D-specific scFv antibody fragment, hinge, CH2 and CH3 domains of IgG4, and a C-terminal ErbB2-specific scFv antibody fragment. *In vivo* antitumor activity of the NKAB-ErbB2 antibody in combination with NK cells engineered to express an NKG2D-CAR (NKAR-NK-92 cells) was evaluated in immunocompetent C57/BL6 mice subcutaneously injected with syngeneic GL261/ErbB2 glioblastoma cells that do not express human NKG2D ligands. Right panel: Symptom-free survival of mice either treated by peritumoral injection of NKAR-NK-92 cells alone, or NKAR-NK-92 cells combined with bispecific NKAB-ErbB2 antibody twice per week for 3 weeks.



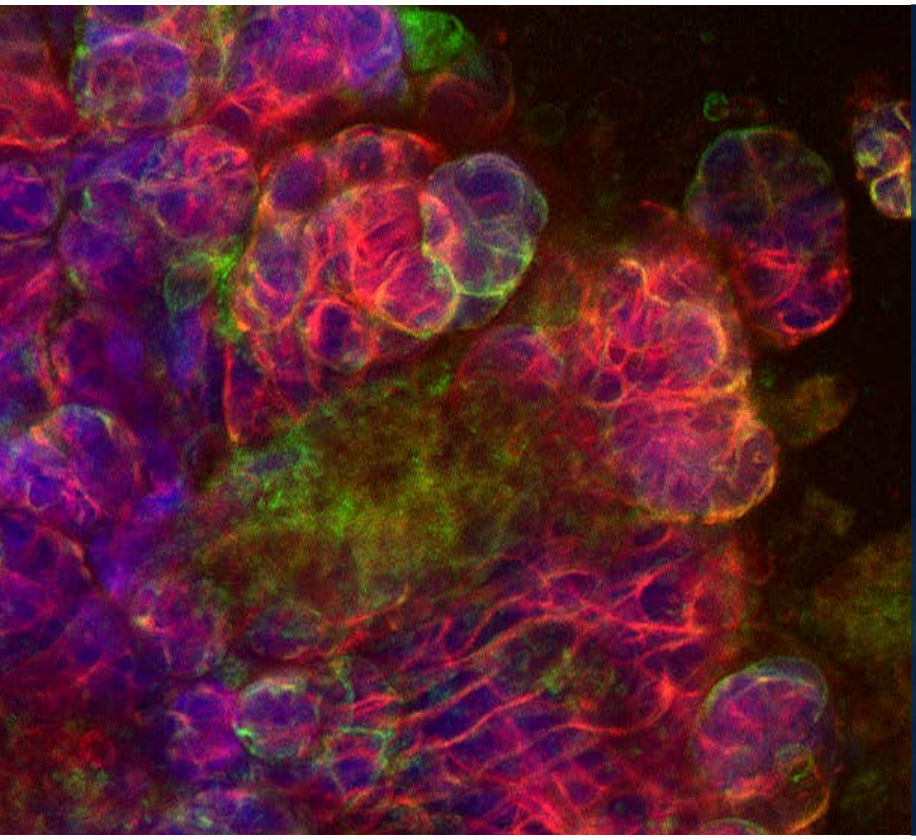
cells transduced with an NKG2D-CAR vector, we observed targeted cell killing and greatly enhanced antitumor activity, which was retained *in vivo* in a preclinical mouse tumor model (Fig. 1). Hence, this combination strategy represents a powerful approach to simultaneously enhance tumor-antigen-specific as well as NKG2D-CAR and natural NKG2D-mediated cytotoxicity, which may be particularly useful to target tumors with heterogeneous target antigen expression.

CAR-NK cells for clinical applications

To assess safety and antitumor activity of CAR-NK cells in a clinical setting, together with colleagues from the Institute for Neurooncology, the Department of Neurosurgery and the German Red Cross Blood Donation Service in Frankfurt we are conducting a phase I clinical trial of intracranial injection of the clonal ErbB2-specific CAR NK-92 cell line NK-92/5.28.z in patients with recurrent ErbB2-positive glioblastoma (CAR2BRAIN; NCT03383978, clinicaltrials.gov). No dose-limiting toxicities were encountered in the completed dose escalation part with single dose injection into the wall of the resection cavity during relapse surgery, demonstrating safety and feasibility of our approach. Also treatment of patients of the expansion cohort has recently been concluded, with follow-up ongoing. These patients received additional weekly injections of NK-92/5.28.z cells through an implanted catheter and reservoir. To further enhance the desired stimulatory effect of the CAR-NK cells on endogenous antitumor immunity, the study protocol has now been amended to include an additional cohort of patients scheduled to receive repeated local injections of NK-92/5.28.z cells in combination with a systemically applied immune checkpoint inhibitor. Furthermore, to extend this treatment approach to other ErbB2-expressing cancers such as breast carcinoma and non-small cell lung carcinoma, we are testing the activity of NK-92/5.28.z cells in respective preclinical models.

Modulation of the tumor microenvironment by CAR-engineered NK cells

In addition to direct killing of tumor cells, CAR-NK cells can contribute to tumor control by recruitment of and cross-talk with other immune cells through cytokines and chemokines secreted after effector cell activation. In immunocompetent glioblastoma mouse models, treatment of syngeneic murine tumors expressing human ErbB2 with ErbB2-specific NK-92/5.28.z cells induced endogenous humoral and cellular antitumor immune responses resulting in tumor rejection and long-term protection of the animals against tumor rechallenge. In ongoing work we investigate means to further enhance this immunostimulatory effect of CAR-NK cells through modulation of their cytokine profile. One such approach is based on the expression of an immunocytokine which harbors a PD-L1-specific antibody domain fused to an IL-15 superagonist. Secretion of this molecule by CAR-NK cells and retention within the tumor microenvironment (TME) by binding to PD-L1 on cancer cells can simultaneously block the PD-1/PD-L1 immune checkpoint and provide a high local concentration of the cytokine to support antitumor activity of CAR-NK and bystander immune cells. Likewise, blockade of immunoregulatory IL-10 that is secreted by activated CAR-NK cells may enhance pro-inflammatory activity in the TME (Fig. 2).



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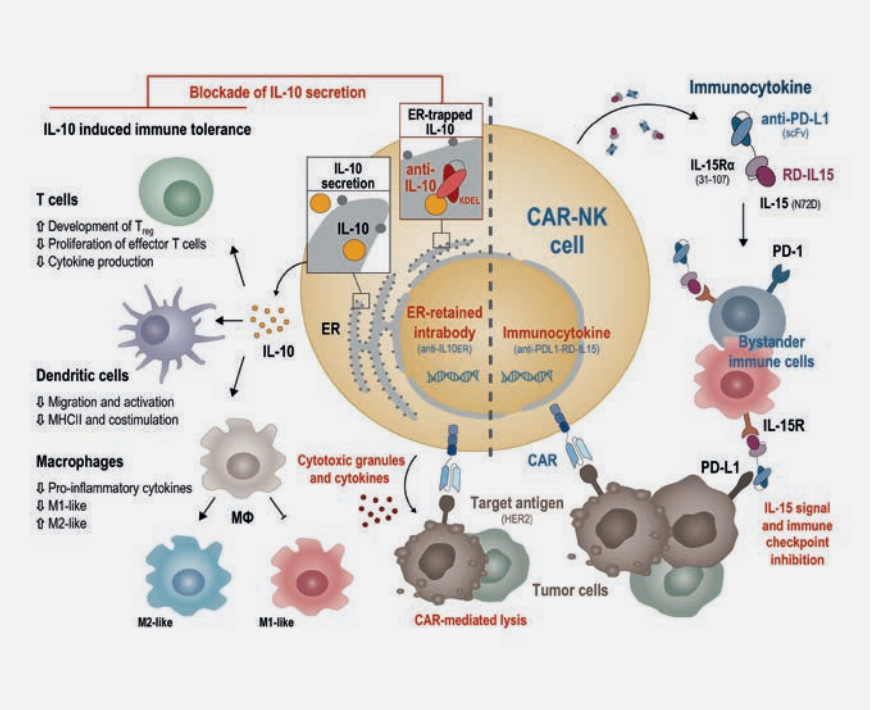

Figure 2.
Modulation of the tumor microenvironment by genetically engineered NK cells. In immunocompetent mouse glioblastoma models, clonal ErbB2-specific NK-92/5.28.z CAR-NK cells induced endogenous antitumor immunity resulting in cures and long-term protection against rechallenge. To overcome immunosuppressive effects of the tumor microenvironment and further enhance the immunomodulatory activity of the CAR-NK cells, we generated derivatives of NK-92/5.28.z cells in which we either blocked secretion of immunoregulatory IL-10 with an endoplasmic reticulum-retained intracellular IL-10 antibody (anti-IL10ER), or expressed a PD-L1-targeted IL-15 immunocytokine (anti-PDL1-RD-IL15) to simultaneously block the PD-1/PD-L1 immune checkpoint and provide IL-15 activity to surrounding immune cells. Preclinical testing of these derivatives is ongoing.

Zentrale Einheit Transgenic Core / Genome Regulation

Transgenic Core Facility / Genome Regulation

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Genetically engineered mouse models (GEMMs)

lncRNAs

genome editing

biomedical mouse models

Recent advances in genome-editing, in particular the CRISPR/Cas9 technology, have revolutionized the generation of mouse models and Knock-out (KO) mouse models in particular. Combination of CRISPR with homology-directed targeting for insertion of short sequences directly into the genome (Knock-in, KI) opens novel opportunities in generating biomedical relevant mouse models. In addition, novel approaches for targeted and reversible gene inactivation will further expand the possibilities of generating mouse models for biomedical research. The TCF also offers strain conservation and rederivation systems to import new mouse strains from other researchers.

The design of the desired genome modification is the fundamental first step in generating any GEMM and a most relevant biomedical mouse model. The desired genetic modification should evoke minimal damage to the normal function of the genome with minimal to none off-target effects. To achieve this a deep knowledge of the genome is required. A complex integration of gene editing techniques is the important next step

Die Transgenic Core Facility / Genome Regulation (TCF) am Georg-Speyer-Haus generiert neue und innovative Mausmodelle für die Krebsforschung durch den Einsatz neuester Technologien der Genommodifikation. Ende 2021 hat Dr. Phillip Grote die Leitung der TCF übernommen. Er bringt seine langjährige Erfahrung in der Entwicklung von transgenen Mausmodellen mit und seine Expertise in der Analyse einer neuen Klasse von Genen, den langen, nicht-protein kodierenden RNAs (lncRNAs), die auch in der Krebsforschung ein immer größeres Interesse für mögliche Therapieansätze wecken.
Die TCF hat ein starkes Portfolio an etablierten Systemen um biomedizinisch relevante Mausmodelle zu etablieren. Dazu gehört die Transgen-generierung mithilfe aktuellster Versionen der CRISPR/Cas9 Genschere, die es nicht nur erlaubt, Bereiche des Genoms einfach zu entfernen, sondern auch kurze Abschnitte gezielt zu integrieren, um krankheitsrelevante Mutation einzubringen, bestimmte Zelltypen zu markieren oder Gene für eine spätere, genetische Inaktivierung in z.B. Tumoren zu markieren. Um einen robusten Ablauf sicherzustellen, hat die TCF Techniken zur Transgen-generierung weiter optimiert und hochwertige Protokolle weiterentwickelt. So kam im Jahr 2022 auch die Möglichkeit dazu, anstatt Gene einfach auszuschalten, stattdessen die Proteine die von manchen dieser Gene generiert werden, pharmakologisch verschwinden zu lassen, was oft zielgenauer und schneller als klassische Verfahren ist.
Der Genklasse der lncRNAs kommt eine immer größere Bedeutung bei der Regulation des Genoms zu. LncRNA Gene sind zelltypspezifischer exprimiert als herkömmliche proteinkodierende Gene und können daher spezifischer manipuliert werden; sie sind daher interessante Zielmoleküle für eine pharmakologische Intervention. Durch gezielte genetische Veränderungen in Mausmodellen, konnten wir bisher einige dieser lncRNAs ausschalten und dadurch eine wichtige Rolle bei der Genregulation zeigen. Da die Genklasse der lncRNAs noch relativ neu ist, wird es in den nächsten Jahren wichtig werden, wie diese in der Zelle genau funktionieren, um neue Ansätze für Therapien auf der Basis der lncRNAs zu entwickeln.

in the GEMM generation process. The constant development of novel applications for the CRISPR system expands the toolbox for gene editing rapidly and any relevant new tool is imported to the TCF.

The current toolbox of the TCF allows *ab initio* development of simple gene knockouts by deletion approaches (Fig. 1A), point mutations, patient alleles, precise small and large deletions (>10kb), and protein truncations. These approaches are usually quite fast to deliver mouse models within 3-6 months. The next level approach is to employ CRISPR/Cas9 to allow targeted integration of short (+/- 1kb) genetic elements. This can allow the tagging of proteins of interest or marking a gene of interest for subsequent deletion by a CRE recombinase driver line (Fig. 1B). Such approaches require prior optimizations and will usually require 4-8 months. Standard targeting approaches for complex transgenes can take from 6-18 months (Fig. 1C). The expansion of the TCF toolbox with additional mouse strain development will allow to establish targeted degradation approaches for any protein of interest. In these systems

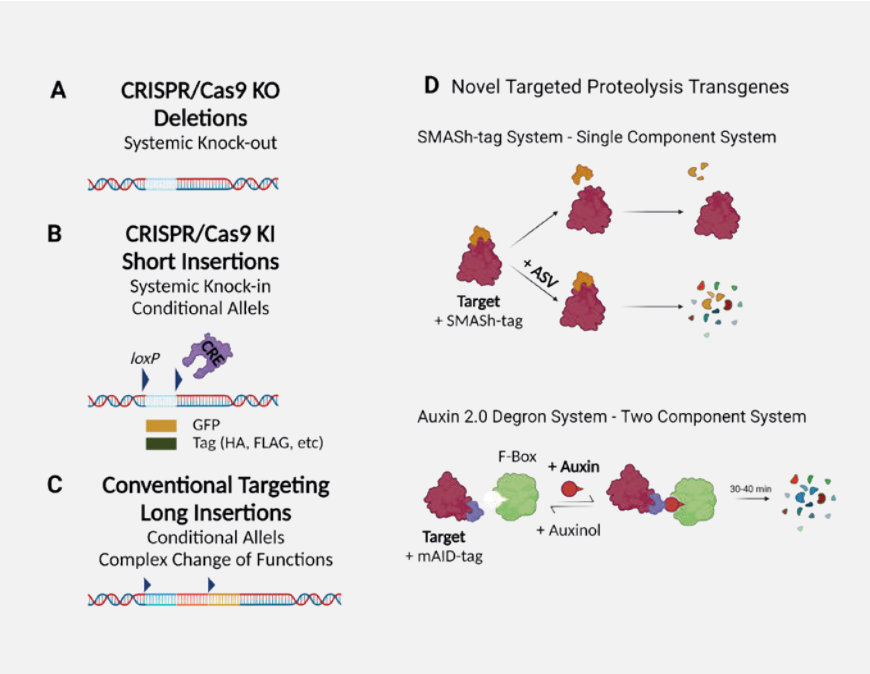
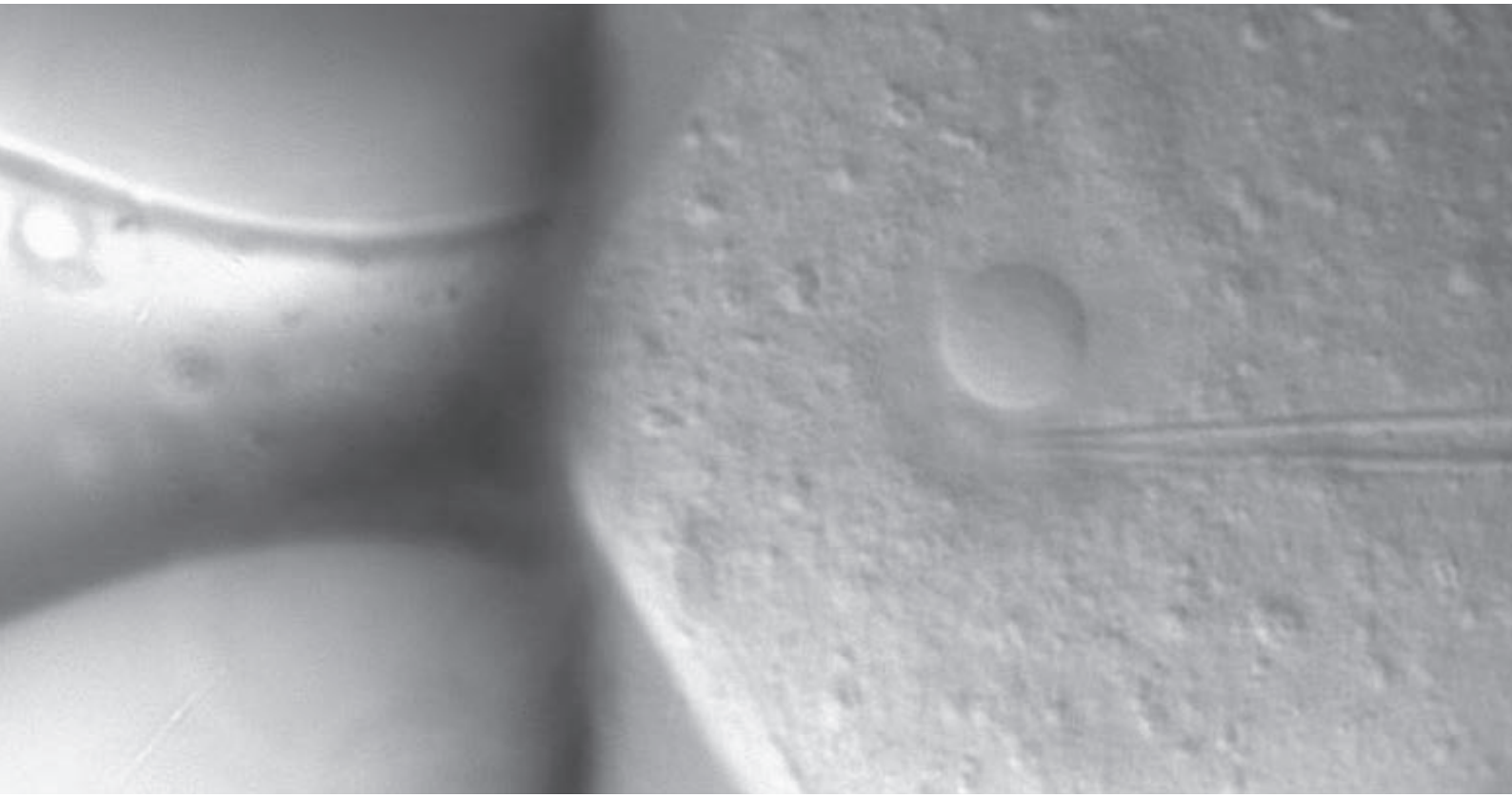


Figure 1.
(A) CAS9 directed gene deletion from several basepairs up to the megabase range that is usable for all techniques shown in Fig. 2. (B) CAS9 assisted integration of short genetic elements specific at any site of the genome. This approach is also available for all techniques shown in Fig. 2. (C) Complex transgenes require the modification of embryonic stem cells prior to mouse generation (Fig. 2 top two techniques). (D) The novel transgenic approaches of drug-inducible targeted protein degradation are shown as an example and are currently under development at the TCF. The SMASh-tag either removes itself and gets degraded or, when the drug Asunaprevir (ASV) is present, drags the attached protein along for degradation. The Auxin system is derived from plants and allows the degradation of the tagged protein of interest when the Auxin drug is present (administered) and the F-box protein (OsTIR1) is available. The second component (OsTIR1) makes this system versatile as this can be directed only to be present in certain cell types of interest.



a protein of interest is tagged (see Fig. 1B) with short peptide that can bind a small molecule drug. The binding of this drug can induce protein degradation and removal of the drug from the mouse does allow the reappearance of the protein (Fig. 1D). These novel approaches do open up new possibilities for biomedical research.

The further development of the CRISPR toolbox also allows to setup novel methods for the generation of transgenic mice. Current protocols require the presence of wild type embryos that serve as a host for transgenic embryonic stem cells or to be modified by CRISPR/Cas9 *ex vivo* (Fig. 2). Novel techniques employing CRISPR/Cas9 now allow the direct genetic modification of wild type embryos in the host mother animal, without the need so sacrifice them (i-GONAD) (Fig. 2, bottom right). This novel technique will reduce the number of required animals for generating mouse models drastically and thereby strictly follows the international guidelines for animals experiments to reduce animal numbers when possible. In addition, the process of generating novel mouse lines is faster for simple CRISPR/Cas9 KO or KI (Fig. 1A,B) and only takes 2-3 months. The genome regulation group analyses the *in vivo* function of the novel gen category of long non-protein coding RNAs (lncRNAs), which are as abundant as protein coding RNAs in the genome. These lncRNAs can participate in the fine tuning of gene regulation and thereby are important modifiers of genome activity and outcome. We could show previously that the activity of the lncRNA *Hands-down* is essential for embryo development by adjusting the expression levels of its nearby protein coding gene. Another

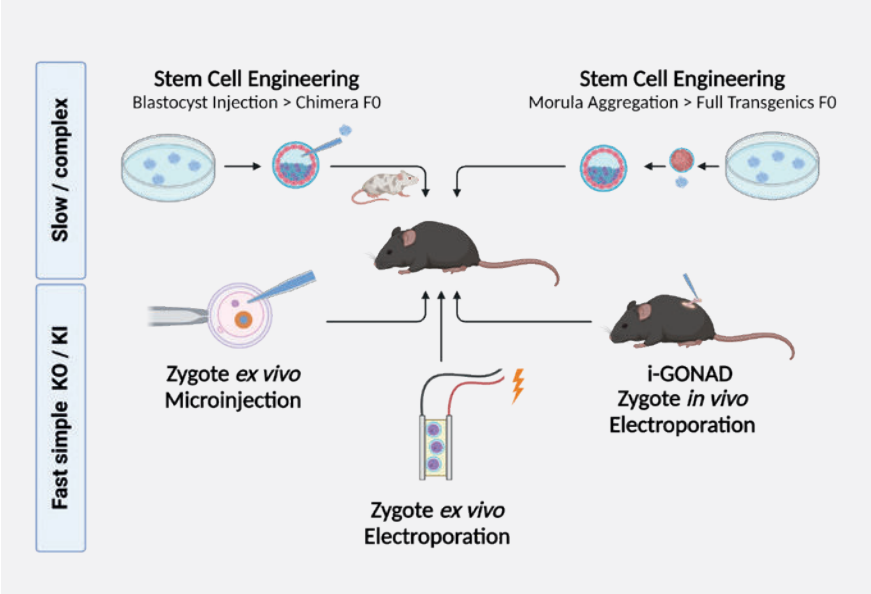
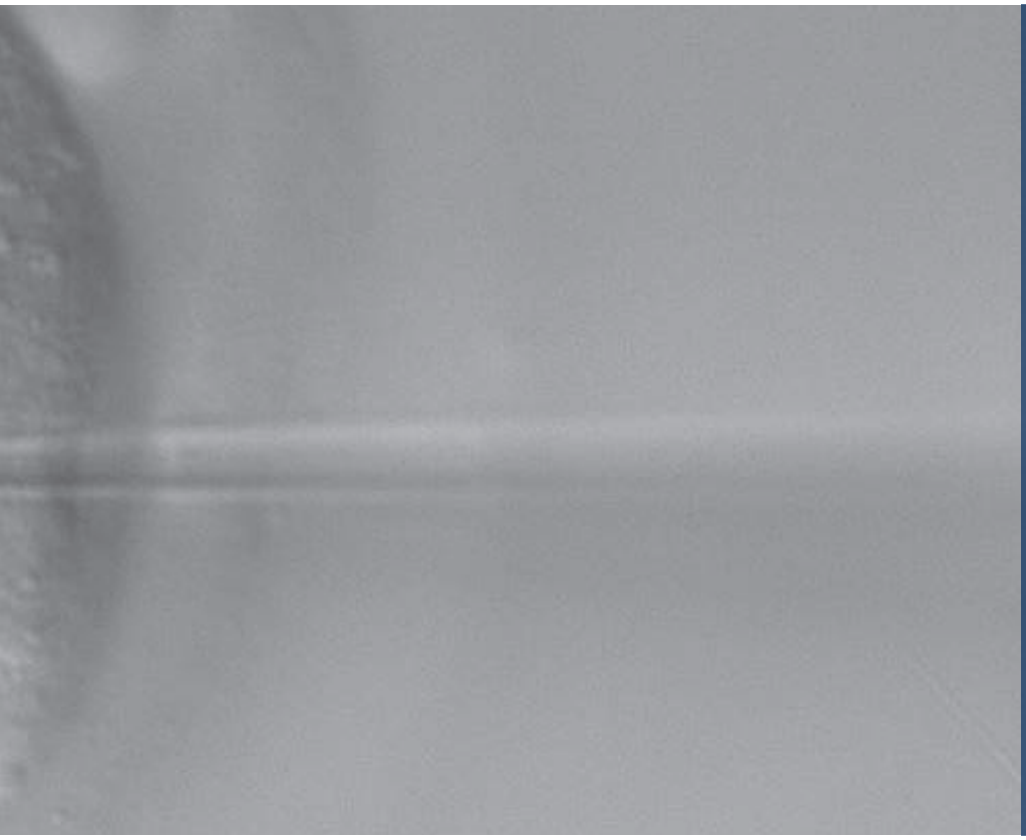


Figure 2.
Techniques for the generation of transgenic mice available at the TCF. The novel approach i-GONAD is currently under development and will be available soon as a service from the TCF.



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lncRNA, which is currently under investigation is the lncRNA *cPlatr26*, which we can show that it regulates the alternative splicing of its neighboring protein coding gene *Itga6* (Fig. 3), which is not only important in heart development but also an important marker for tumor metastasis.

Figure 3.
The lncRNA *cPlatr26* regulates alternative splicing of the Integrin coding gene *Itag6*. (A) predicted RNA structure of *cPlatr26*. (B) Mouse embryos that lack *cPlatr26* show aberrant splicing of the *Itag6* RNA so that the splice variant *Itga6A* is nearly eliminated in the developing heart.

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Akademische Ausbildung
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2022-2023 Theresa Krack
2022-2022 Lara Paulini
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2021-2021 Celina Reiter
2020-2021 Bianca Gregorz
2020-2021 Alessia Cais
2019-2020 Nathalie Thomasberger

Doktorarbeiten
Costanza Zanetti
Christina Karantanou
Raquel S. Pereira

AG Medyouf

Saini SK, Holmberg-Thydén S, Bjerregaard AM, Unnikrishnan A, Dorfmüller S, Platzbecker U, Tirado-Gonzalez I, Bönig H, El Fassi D, Grønbæk K, Pimanda J, Medyouf H, Hadrup SR.
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*equal contribution
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Akademische Ausbildung
Doktorarbeiten

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Masterarbeiten

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AG Arkan

Nicolas AM, Pesic M, Engel E, Ziegler PK, Diefenhardt M, Kennel KB, Buettner F, Conche C, Petrocelli V, Elwakeel E, Weigert A, Zinoveva A, Fleischmann M, Haupl B, Karakutuk C, Bohnenberger H, Mosa MH, Kaderali L, Gaedcke J, Ghadimi M, Rodel F, Arkan MC, Oellerich T, Rodel C, Fokas E, Greten FR (2022)
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Akademische Ausbildung
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AG Farin

Krämer A, Amelie Tjaden T, Ndreshkjana B, Tredup C, Farin HF, Knapp S, Janin YL, Müller S.
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Niklas Schött
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Adele Nicolas / PhD
"Inflammatory Cancer Associated Fibroblasts Decisive Role in Therapy Resistance Among Rectal Cancer Pations"
Promotion im Fachbereich 15 Biowissenschaften, Goethe Universität Frankfurt

AG Sevenich

Strecker MI, Wlotzka K, Strassheimer F, Roller B, Ludmirski G, König S, Röder J, Opitz C, Alekseeva T, Reul J, Sevenich L, Tonn T, Wels WS, Steinbach JP, Buchholz CJ, Burger MC
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Akademische Ausbildung
Woon Hyung Chae
"Evaluating Magnetic Resonance Spectroscopy as a tool for monitoring therapeutic response of whole brain radiotherapy in a mouse model for breast-to-brain metastasis". Promotion im Fachbereich 16 Medizinische Fakultät, Goethe Universität Frankfurt

AG Wels

Strecker MI, Wlotzka K, Strassheimer F, Roller B, Ludmirski G, König S, Röder J, Opitz C, Alekseeva T, Reul J, Sevenich L, Tonn T, Wels WS, Steinbach JP, Buchholz CJ, Burger MC.
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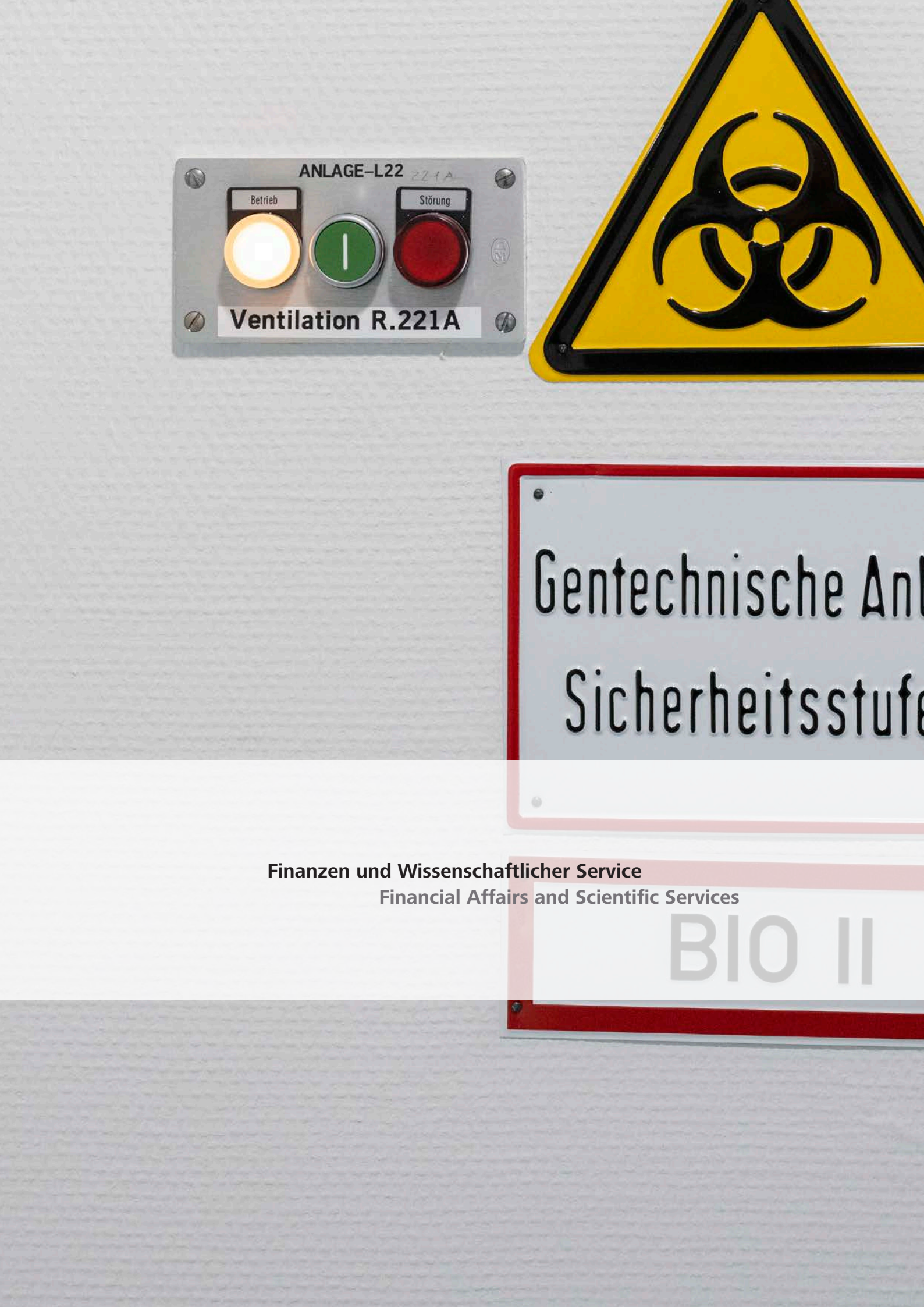

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Der Bereich setzt jährlich ein Finanzvolumen von etwa 10 Millionen Euro um und betreut dabei rund 100 Mitarbeiter:innen. Sie wird von Franziska Hasslinger-Pajtler geleitet, die dabei von Robert Dornberger unterstützt wird. Besonderer Fokus der Administration liegt neben den klassischen Kernaufgaben einer jeden Verwaltung auf den Themen Compliance, Datenschutz, Digitalisierung & Forschungsdatenmanagement, Diversity, Nachhaltigkeit, Personalbindung und -entwicklung sowie der Vereinbarkeit von Beruf und Familie.
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Adrian Gresik ist verantwortlich für die vielfältigen Aufgaben des Innendienstes und koordiniert gemeinsam mit Hana Kunkel die anspruchsvollen Umbau- und Sanierungsvorhaben des Instituts. Das Team des Innendienstes mit Krzysztof Data, Heinrich Krompiec und Michael Paul kümmert sich primär um die Gebäudetechnik und unterstützt zudem bei der Organisation von wissenschaftlichen Tagungen und Veranstaltungen. Ansprechpartner in der Telefonzentrale und am Empfang ist Bernd Würdemann.
Yoseph Alazar, Yasemin Piskin, Pulpan Lacramioara und Neriman Sarac sind für die Laborreinigung, die Abfallentsorgung sowie die Laborbedarfsversorgung verantwortlich.

The Finance/Administration department with its yearly budget of approximately 10 million Euros manages around 100 staff members. Since September 2019 it has been headed by Franziska Hasslinger-Pajtler, who is supported by Robert Dornberger. In addition to the classic core tasks of any administration, the Administration Department is currently focusing in particular on the topics of compliance, data protection, digitization & research data management, diversity, sustainability, staff retention & development and work-life balance. Giuseppina Virgillito in the HR office handles all HR-related issues. Ilka Grau's focus in financial accounting is on managing DFG funding and federal project funding. Luca Fabisch is in charge of accounts payable and Lars Fischer supports both with various tasks in financial accounting. Brigitte Huth processes the travel expense reports of the house. Sabine Finger prepares the balance sheet and Christof Kaiser processes the third-party funds of the State of Hesse. Dr. Alina Jurcoane is the contact person for all matters concerning third-party funds from the European Union. Belinda Gehrmann assists in the team around the commercial management and also supports the personnel office. Dr. Klaus Cußler is the contact person for the museum in the Georg-Speyer-Haus and historical issues. Adrian Gresik is responsible for the diverse tasks of the Building Services department and coordinates the demanding reconstruction and renovation projects in cooperation with Hana Kunkel. The Building Services department team with Krzysztof Data, Heinrich Krompiec and Michael Paul primarily takes care of the building services, various constructions and installations, and also supports the organization of scientific conferences and events. The contact person in the switchboard and at the reception is Bernd Würdemann. Yoseph Alazar, Yasemin Piskin, Pulpan Lacramioara and Neriman Sarac are responsible for laboratory cleaning, proper waste disposal and recycling, and laboratory supplies.

Wissenschaftlicher Service

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Tierhaltung am Georg-Speyer-Haus

Am Georg-Speyer-Haus wird eine Vielzahl unterschiedlicher Mausmodelle gezüchtet und in genehmigten Experimenten eingesetzt. Die Tierhaltung erfüllt hierbei alle aktuellen gesetzlichen Anforderungen und steht im Einklang mit der europäischen Verordnung 2010/63/EU sowie den deutschen Gesetzen zum Schutz der für wissenschaftliche Zwecke verwendeten Tiere. Die Tierpflege und alle wissenschaftlichen Arbeiten orientieren sich grundsätzlich am ethischen „3R" Prinzip des Verringerns, Verbesserns und Vermeidens von Tierversuchen. Alle Mitarbeiter sind erfahren im Umgang mit Mäusen und konsequent dem Tierwohl verpflichtet. Fortlaufend geschultes und sachkundiges Tierhaltungspersonal ermöglicht eine gute und umfassende Pflege der Tiere sowie wissenschaftliche Assistenz bei der Durchführung der Versuche.

In der GSH Tierhaltung können unterschiedliche Bildgebungsverfahren eingesetzt werden. Beispielsweise verfügt das Institut über einen 7 Tesla Hochleistungstomographen (PharmaScan 7T, Bruker Biospin) für anatomische, funktionelle und metabolische Untersuchungen sowie ein modernes *in-vivo* Bildgebungsverfahren (IVIS Lumina II, PerkinElmer) für Bioluminescenz- und Fluoreszenzanalysen in kleinen Nagetieren. Das SARRP System (Small Animal Radiation Research Platform, Xstrahl Medical) bietet Möglichkeiten der therapeutischen Strahlentherapie bei Mäusen. Weitere Geräte erlauben eine Ganzkörperbestrahlung kleiner Nagetiere (BioBeam, Gamma Medical) und die Generierung sogenannter „humanisierter" Mausmodelle. Mit Hilfe einer endoskopischen Apparatur (Coloview, Karl Storz) können Darmspiegelungen bei der Maus durchgeführt werden beziehungsweise konfokale endomikroskopische Untersuchungen der Darmschleimhaut (Cellvizio, Mauna Kea Technologies) erfolgen.

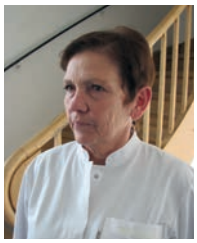
Aufgrund der zurückliegenden Corona Beschränkungen und der aktuellen Energiekrise wurde der GSH Tierhaltungsbereich im Jahr 2022 neu strukturiert. Der Zuchtcontainer wurde nach 16 Jahren intensiver Nutzung geschlossen und alle Tiere wurden im Hauptgebäude zusammengeführt.

Animal Husbandry at the Georg-Speyer-Haus

Our Animal Facility is designed and run in line with the recent legislation and meets all requirements of the directive 2010/63/EU on animal welfare and the German law on the protection of animals used for scientific purposes and consistently adheres to the ethical „3R" principle of reduction, refinement and replacement. All scientists are experienced in laboratory animal care and advised on animal welfare and legal requirements. Ongoing training of qualified staff responsible for animal housing enables a comprehensive service that includes both experienced care and scientific assistance.

The experimental area of the GSH animal facility is equipped with an ultra-high field magnetic resonance imaging system (PharmaScan 7T, Bruker Biospin) for anatomical, functional and metabolic imaging and an *in vivo* imaging system (IVIS Lumina II, PerkinElmer) for quantitative fluorescent and bioluminescent imaging of small rodents. The small animal radiation research platform (SARRP, Xstrahl Medical) provides opportunities for therapeutic treatment that bridge basic research and clinical translation. Other experimental procedures enables whole-body irradiation (BioBeam, Gamma Medical) and creation of variable humanized mouse models. Further scientific research tools are an endoscopic system (Coloview, Karl Storz) for colonoscopic examination and a probe-based *in vivo* confocal laser endomicroscopy platform (Cellvizio, Mauna Kea Technologies).

Because of Covid-19 restrictions and current energy crisis our animal area has been reorganized in 2022. The breeding container has been closed and dismantled after 16 years of usage, all animals were consolidated in the main housing area.



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Zentrale Einheit Histologie
Zur Anfertigung von histologischen Präparaten betreibt das Georg-Speyer-Haus eine Histologie-Serviceeinheit. Hier werden von Frau Petra Dinse, meist automatisiert, die Gewebeaufarbeitung sowie immunohistochemische Färbungen und Standardfärbungen durchgeführt. Weiterhin verfügt das Labor über ein automatisiertes Präparate-Scanner- und Bildanalysesystem, Aperio ScanScope CS2, einen Färbeautomat Leica Autostainer XL sowie einen Leica BOND max zur Anfertigung von automatisierten Immunfärbungen.

Core Facility Histology
The Georg-Speyer-Haus operates a histology core facility. Petra Dinse is responsible for the mostly automated procedures of tissue processing and immunohistochemistry as well as hematoxylin / eosin staining. The laboratory is equipped with a slide scanner and image analysis system, Aperio ScanScope CS2, a Leica AutostainerXL and a Leica BOND max for automated immunostaining.

Dr. Stefan Stein
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Zentrale Einheit Durchflusszytometrie (FCU)
Die zentrale Durchflusszytometrie-Einrichtung (FCU) betreut drei Geräte zur Zellanalyse (BD LSRFortessa, BD FACSCantoII, Cytek Aurora) und zwei Zellsorter (BD FACSAriaI, BD FACSAria Fusion). Zusätzlich werden in der Einrichtung ein Bioplex200 zur Multiplex-Analyse und ein ABC Blood Counter zur Blutzellanalyse betrieben. Geleitet wird die Serviceeinheit von Dr. Stefan Stein, der auch Ansprechpartner für allgemeine Fragen zur Durchflusszytometrie und bei der Entwicklung und Anpassung neuer Mess- und Sortieransätze ist. Unterstützt wird er hierbei durch Frau Annette Trzmiel, die die anfallenden Hochgeschwindigkeits-Zellsortierungen durchführt und für den einwandfreien Zustand aller Durchflusszytometrie-Geräte am Institut verantwortlich ist. In einigen Fällen fungiert Herr Thorsten Geyer als zusätzlicher Operator an den Zellsortern.

Flow Core Unit (FCU)
The Flow Core Unit (FCU) of the Georg-Speyer-Haus operates three flow cytometer instruments (BD LSRFortessa, FACSCantoII, Cytek Aurora) and two cell sorters (BD FACSAriaI and BD FACSAria Fusion). In addition, the facility runs a Bioplex200 for multiplex analysis and an ABC Blood Counter for blood cell analysis. Dr. Stefan Stein oversees the performance of the core facility and is available for scientific questions regarding flow cytometry in general and the establishment of new flow based assays. He is supported by Annette Trzmiel who is responsible for high-speed cell sorting as operator for all research groups. Annette also takes care of the maintenance and functionality of the flow cytometers in the institute. Occasionally, Thorsten Geyer serves as an additional sorting operator.

Dr. Tijna Alekseeva
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Zentrale Einheit Mikroskopie und *in vivo* Imaging

Die Imaging Core Facility bietet Zugang zu den hochentwickelten Bildgebungsinstrumenten im GSH, zu denen derzeit ein 7T-Kleintier-MRT (Bruker), ein konfokales Spinning-Disc Mikroskop (CQ1, Yokogawa), ein konfokales Mikroskop (SP5, Leica), Fluoreszenz-/ Lichtmikroskope (AxioImager, Zeiss) sowie Bildverarbeitungsstationen (CQ1- Bildanalyse und CellPathFinder) gehören. Verantwortlich für die Schulung, Koordination und Wartung ist Dr. Tijna Alekseeva. Sie dient als zentrale Anlaufstelle für alle Fragen im Zusammenhang mit den Bildgebungsgeräten sowie für die Beratung zum Versuchsaufbau, zur Entwicklung neuer Analyseansätze und zu allgemeinen Fragen im Zusammenhang mit der Bildgebung. Am MRT wird Dr. Alekseeva in Teilzeit von Marco Lollies unterstützt, der sich um Routinemessungen für interne und externe Nutzer kümmert.

Imaging Core Facility

The Imaging Core Facility provides access and training to the sophisticated imaging instruments in the GSH, currently including a small animal 7T MRI (Bruker), spinning disc confocal microscope (CQ1, Yokogawa), confocal microscope (SP5, Leica), fluorescent/light microscopes (AxioImager, Zeiss) as well as image processing stations (CQ1 image analysis and CellPathFinder).
Responsible for the training, coordination and maintenance is Dr. Tijna Alekseeva. She serves as single point of contact for all questions related to the imaging equipment as well as advice on the experimental set up, development of new analytical pipelines and general enquiries related to imaging.
At the MRI, Dr. Alekseeva is supported part time by Marco Lollies who is taking care of routine measurements for internal and external users.
All services are available to GSH staff and external users.

Steffen Luft
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IT
Steffen Luft leitet als Chief Information Officer (CIO) die IT. Er nimmt die zentralen Tätigkeiten der Unterstützung der Mitarbeiterinnen und Mitarbeiter des Hauses in allen Fragen der IT, der Serverbetreuung, des IT-Projekt-Managements, der Netzwerkadministration und des Einkaufs wahr.

IT
Steffen Luft is Chief Information Officer (CIO) of the Institute. His main tasks are the maintenance of the servers, IT project management, administration of the networks, and the support of the colleagues in the institute.

Dr. Stefan Stein
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Geräte und Biologische Sicherheit
Dr. Stefan Stein berät bei der Beschaffung der nötigen wissenschaftlichen Arbeitsgeräte. Außerdem kümmert er sich als Beauftragter für biologische Sicherheit um die Arbeitssicherheit und ist zuständig für die Kommunikation mit den entsprechenden Aufsichtsbehörden.

Devices and Biological Safety
Dr. Stefan Stein attends for lab equipment and devices. As biosafety officer, he is responsible for biological safety at the institute and for communication with respective authorities.

Dipl. Biol. Thorsten Geyer
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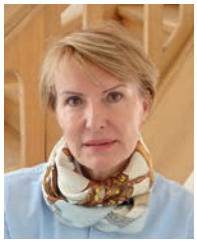
Arbeitssicherheit und Strahlenschutz
Thorsten Geyer ist am Institut die Fachkraft für Arbeitssicherheit und berät die Mitarbeiter und verantwortlichen Gruppenleiter in allen Belangen des betrieblichen Arbeitsschutzes. Als Strahlenschutzbeauftragter ist er für die Organisation der entsprechenden Einrichtungen und die Kommunikation mit den Aufsichtsbehörden zuständig.

Occupational safety and radiation protection
Thorsten Geyer is the specialist for occupational safety at the institute and advises the employees and responsible group leaders in all matters relating to occupational health and safety. As radiation protection officer, he is responsible for organizing the relevant facilities and communicating with the supervisory authorities.



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Hygiene- und Labormanagement
Hana Kunkel achtet auf die Einhaltung der geltenden Laborstandards und Arbeitssicherheitsbedingungen. Sie verantwortet den Spülküchenbereich und koordiniert die Reinigungsdienstleistungen. Darüber hinaus ist Sie Ansprechpartnerin der Gruppenleitungen für die Laborplanung und Laborausstattung.

Facility- / Lab Management
Hana Kunkel ensures compliance with the applicable laboratory standards and work safety regulations. She is responsible for the scullery area and coordinates all cleaning services. In addition, she is the contact person for all group leaders concerning laboratory planning and laboratory equipment.

Der Verein „Freunde und Förderer des Georg-Speyer-Hauses“

The Association “Friends and Sponsors of the Georg-Speyer-Haus”

Innovative Forschung und wissenschaftlicher Fortschritt in unserer Gesellschaft sind nur möglich durch das Engagement der Wissenschaftler/innen und die aktive Unterstützung von Forschungsförderern aus Öffentlichkeit, Wissenschaft und Wirtschaft. Diesem Engagement hat sich der Verein „Freunde und Förderer des Georg-Speyer-Hauses“ verpflichtet: Sein Ziel ist es, über die Grundfinanzierung durch Bund und Länder hinaus für weitere erforderliche Mittel zu sorgen und so das hohe Niveau der Grundlagenforschung zu sichern. Mitglied im Verein kann werden, wer den wissenschaftlichen Fortschritt im Bereich der Krebsforschung und der experimentellen Therapie zum Wohle der Allgemeinheit fördern möchte und Interesse hat am Forschungsprozess und am Diskurs über Ergebnisse und deren Nutzen für die Allgemeinheit. Neben der einfachen Mitgliedschaft (Freund/innen) und der Forschermitgliedschaft (Wissenschaftler/innen, Student/innen) besteht die Möglichkeit der fördernden Mitgliedschaft für Einzelpersonen oder Firmen. Förderer können im Jahrbuch und auf der Spendentafel aufgeführt werden.

Da der Verein eine gemeinnützige Einrichtung ist, sind Mitgliedsbeiträge und Spenden im Rahmen der zulässigen Höchstbeträge von der Steuer absetzbar.

Innovative research and scientific advances are only possible through generous financial support from public and private sponsors. The association „Friends and Sponsors of the Georg-Speyer-Haus“ has committed itself to this task. The major goal of the association is to raise the necessary funds to supplement the basic financing provided by the federal and state governments. This should ensure a continuing high quality of basic research. Everybody who would like to support research in the fields of cancer and experimental therapy is welcome to join the association. Private persons can become supporting members (“friend“) or research members (scientists and students). Moreover, private individuals and companies may obtain corporate membership. Sponsors will be listed in both the annual report and the table of benefactors in the Institute.

Since the association is a non-profit organisation, all membership fees and donations are tax deductable.

Jährliche Mitgliedsbeiträge
Annual membership fees

Forschermitglied
Scientist
100,– €

Studenten
Students
12,– €

Freund
Friend
150,– €

Förderer
Sponsor
1000,– €

Firmenmitgliedschaft
Company membership
5000,– €

FREUNDE UND FÖRDERER DES
GEORG SPEYER HAUSES E.V.

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„Wichtig ist,
dass man nicht aufhört
zu fragen.“
Albert Einstein