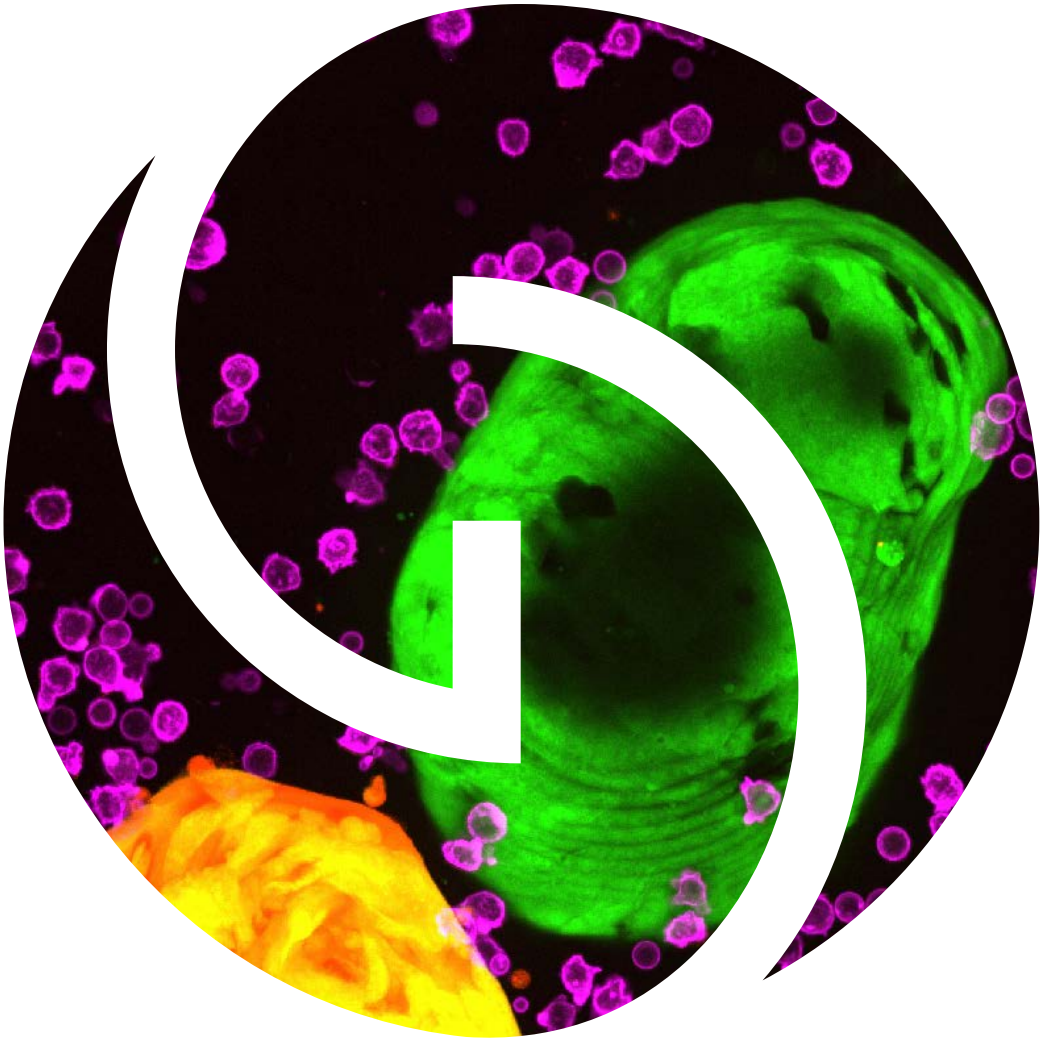
GEORG SPEYER HAUS
INSTITUT FÜR TUMORBIOLOGIE
UND EXPERIMENTELLE THERAPIE



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

Annual Report Georg-Speyer-Haus 2020

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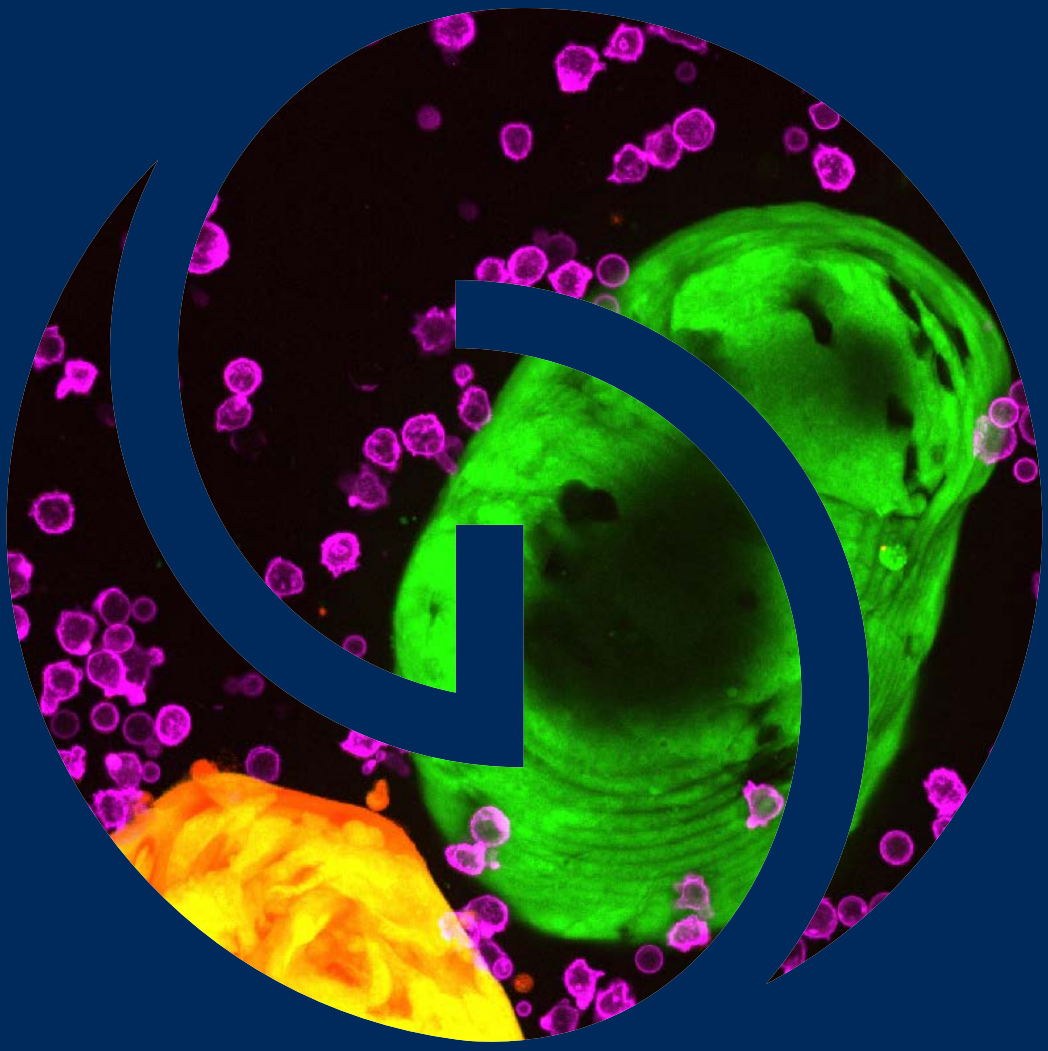
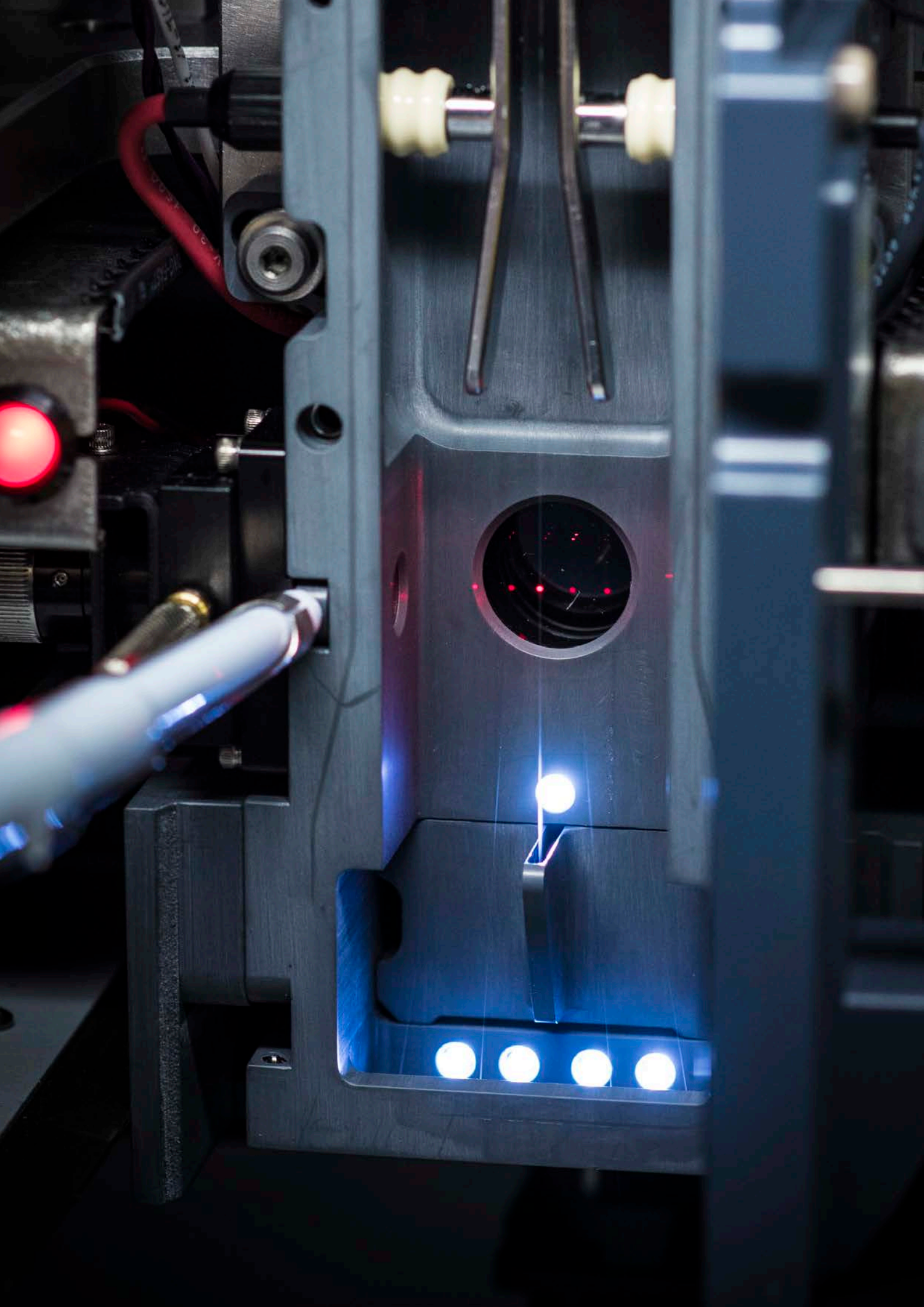
Die Grundfinanzierung des Georg-Speyer-Hauses wird vom Bundesministerium für Gesundheit und dem Hessischen Ministerium für Wissenschaft und Kunst getragen.

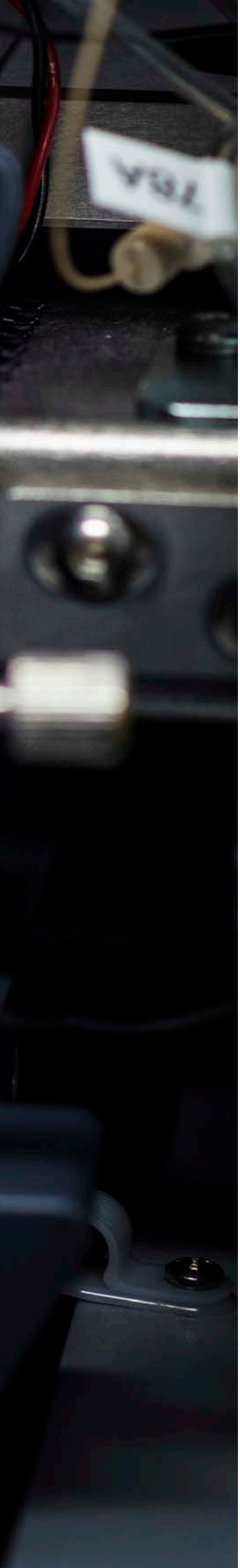
The basic funding of the Georg-Speyer-Haus is provided by the Federal Ministry of Health and the Ministry of Higher Education, Research and the Arts of the State of Hessen.

Forschen für das Leben

Research for Life







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

2020 stand und steht im Zeichen der COVID-19 Pandemie und auf Grund der allgemeinen Hygiene- und Abstandsregeln sind die wissenschaftlichen Aktivitäten am GSH derzeit nur eingeschränkt möglich. Für alle Mitarbeiterinnen und Mitarbeiter ist dies ein Jahr der persönlichen und beruflichen Herausforderungen und es ist bemerkenswert, mit welcher Disziplin hier am GSH mit der derzeitigen Situation umgegangen wird.

Im Frühjahr während des ersten Lockdowns konnten wir eine komplette Schließung des Instituts vermeiden und dank des vorbildlichen Engagements aller technischen und administrativen Angestellten sowie unserer Tierpflegerinnen einen Minimalbetrieb aufrechterhalten, so dass die wissenschaftlichen Arbeiten der Arbeitsgruppen im Frühsommer relativ schnell wieder expandiert werden konnten.

Direkte persönliche Kontakte und Reisetätigkeiten wurden wie überall auch bei uns reduziert oder komplett eingestellt, wissenschaftliche Veranstaltungen in den virtuellen Raum verlegt und auch unsere internationalen Sprecher tragen inzwischen per Webinar vor. Selbst wenn in diesen Formaten viel von den Vorzügen der persönlichen Interaktionen auf der Strecke bleibt, so haben wir doch auf der anderen Seite auch einige Vorzüge von Videokonferenzen kennengelernt. Sicherlich werden wir daraus lernen und in Zukunft einiges beibehalten.

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

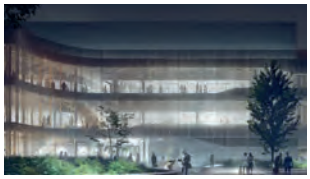
2020 has been and still is dominated by the COVID-19 pandemic. Due to the general hygiene and distancing rules, scientific activities at the GSH are currently possible only to a limited extent. For all employees this is a year of personal and professional challenges and it is remarkable what kind of discipline has been shown at the GSH in the current situation.

In spring, during the first lockdown, we were able to avoid a complete closure of the institute and, thanks to the exemplary commitment of all technical and administrative employees as well as our animal care takers, we were able to maintain minimal operations, so that the scientific work of the Research Groups could be expanded again relatively quickly in early summer.

As anywhere else, direct personal contacts and travel activities have been reduced or completely discontinued, scientific events have been moved to the virtual space and also our international speakers are now giving presentations via webinars. Even if a lot of the advantages of personal interactions are left behind in these formats, on the other hand we have also experienced certain advantages of video conferencing. We will certainly learn from it and keep some of these things in the future.

Sehr erfreulich war der Arbeitsbeginn von Dr. Matthias Ebert, der seit dem Frühjahr unsere Tierhaltung leitet und schon eine Reihe wichtiger struktureller Verbesserungen umsetzen konnte. Seine große Erfahrung in der Leitung verschiedener universitärer Tierhaltungen und seine klar strukturierten Visionen einer modernen State-of-the-Art Haltung sind nicht nur ausgesprochen wertvoll für den laufenden Betrieb, sondern auch für die Planung des neuen Haltungs-und Zuchtbereichs im FCI.

Trotz der massiven Einschränkungen in diesem Jahr hat das GSH sich insgesamt betrachtet sehr positiv weiterentwickelt und insbesondere eine Reihe sehr beachtenswerter wissenschaftlicher Erfolge erzielen können. Der Baubeginn für das FCI steht nun unmittelbar bevor. Dieser Zeit blicken wir gespannt entgegen. Die Berufungsverfahren für zwei LOEWE-FCI Professuren werden voraussichtlich im nächsten Jahr abgeschlossen und wir freuen uns auf die anstehenden Neubesetzungen. Unter Berücksichtigung der positiven Neuigkeiten bezüglich der nahenden Zulassung eines wirksamen Impfstoffes gegen SARS-CoV-2 erhoffen wir uns alle für das nächste Jahr eine Rückkehr zu einer „normalen“ Präsenz am Institut, verbunden mit der Möglichkeit wieder uneingeschränkt zu kommunizieren und sich wissenschaftlich auszutauschen. Daher blicken wir optimistisch nach vorn.



Florian R. Greten, Direktor

The recruitment of Dr. Matthias Ebert in spring as our new head of the animal facility was a great success. He is in charge of our animal husbandry and has already implemented a number of important structural improvements. His vast experience in managing various university animal husbandries and his clearly structured visions of a modern state-of-the-art husbandry are not only extremely valuable for ongoing operations, but also for the planning of the new animal facility of the FCI.

Despite the massive restrictions this year, the GSH has developed very positively overall and has achieved a number of very noteworthy scientific successes in particular. The start of construction for the FCI is now imminent. We look forward to this time with great anticipation. The appointment procedures for two LOEWE-FCI professorships are expected to be completed next year and we are looking forward to the upcoming new appointments. Taking into account the positive news regarding the approaching approval of an effective vaccine against SARS-CoV-2, we all hope for a return to a "normal" presence at the institute next year combined with the possibility of unrestricted communication and scientific exchange. We are therefore looking ahead with great optimism.

Der Baubeginn für das FCI steht nun unmittelbar bevor.

The start of construction for the FCI is now imminent.

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.

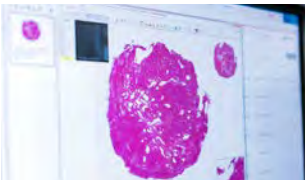
Die laufenden Geschäfte des heutigen Instituts für Tumorbiologie und experimentelle Therapie nimmt der Direktor wahr. Er ist in dieser Tätigkeit dem Stiftungsvorstand verantwortlich. Das Georg-Speyer-Haus ist durch einen Kooperationsvertrag mit der Goethe-Universität Frankfurt verbunden.

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.

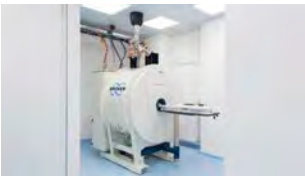


Das Georg-Speyer-Haus wird finanziell vom Bundesministerium für Gesundheit (BMG) sowie vom Hessischen Ministerium für Wissenschaft und Kunst (HMWK) unterstützt. Zusätzlich stehen Mittel aus der Drittmittelförderung öffentlicher und privater Forschungsförderungsorganisationen, aus Kooperationsvereinbarungen 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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FORSCHUNGSBEREICH 1

Zelluläre Kommunikation
in der Stammzellnische

Prof. Dr. D. Krause
Dr. H. Medyouf

Hygiene-
und Labor-
management

H. Kunkel

ZENTRALE EINRICHTUNGEN

Durchflusszytometrie
Histologie
Präklinische Einheit und Bildgebung
Dr. S. Stein
Dr. B. Ritter
Dr. M. Ebert

Transgenic Core Facility
Gene Editing Laboratory
Dr. M. Karimova

FORSCHUNGSBEREICH 2

Zell-Zell Interaktionen
im Tumorstroma

PD Dr. M.C. Arkan
Dr. H. Farin
Prof. Dr. F. R. Greten
Dr. L. Sevenich

VERWALTUNG

Personal, Finanzen,
IT, Innendienst, Einkauf,
Arbeitssicherheit

F. Hasslinger-Pajtler

FORSCHUNGSBEREICH 3

Experimentelle Therapie

Prof. Dr. W. Wels


Falcon

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 are rarely eradicated. Eradication of cancer stem cells in leukaemia or leukaemia stem cells, however, is thought to be important for cure of a cancer.

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 leukaemic stem cells (LSC). The vascular niche, the extracellular matrix, the coagulation system 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 (in collaboration with Prof. S. Dimmeler), hereby, form the basis of our studies.

Trotz verbesserter Therapien, z.B. in Form von Tyrosinkinaseinhibitoren, 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.

Wie bereits von uns und anderen Gruppen publiziert, kann eine gezielte Modulation des Knochenmarksmikromilieus (KMMM), dem Ort, wo eine Leukämie entsteht und voranschreitet, 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, wie spezifische Interaktionen von Leukämiezellen mit der extrazellulären Matrix des KMMMs den Krankheitsverlauf einer Leukämie beeinflussen kann. Ferner sind die Rolle des Blutgerinnungssystems im KMMM, die Adhäsion von Leukämie-induzierenden Zellen im KMMM, die Rolle des Alters des KMMMs und chemische Faktoren im KMMM der Fokus unserer Arbeitsgruppe.

Im Rahmen der Covid-19 Pandemie beschäftigen wir uns auch mit der Rolle von genetischen Faktoren auf den Krankheitsverlauf nach Infektion.

In one project we are studying 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 may be contributory. Indeed, our study has shown that the age of the BMM influences the leukaemia phenotype, at least partially via certain cytokines and their respective receptors. In fact, we demonstrated that high expression of one of these cytokine receptors in paediatric B-ALL may predict central nervous system relapse, offering novel avenues for treatment.

In another project we have shown that the natural anticoagulant system may influence leukaemia progression via a breakdown of the extracellular matrix (ECM). Other proteins in the BMM may favour the establishment of a pro-tumorigenic environment, which accelerates disease progression in acute myeloid leukaemia (AML). Both pathways may be inhibited by a novel use of particular drugs for the treatment of leukaemia.

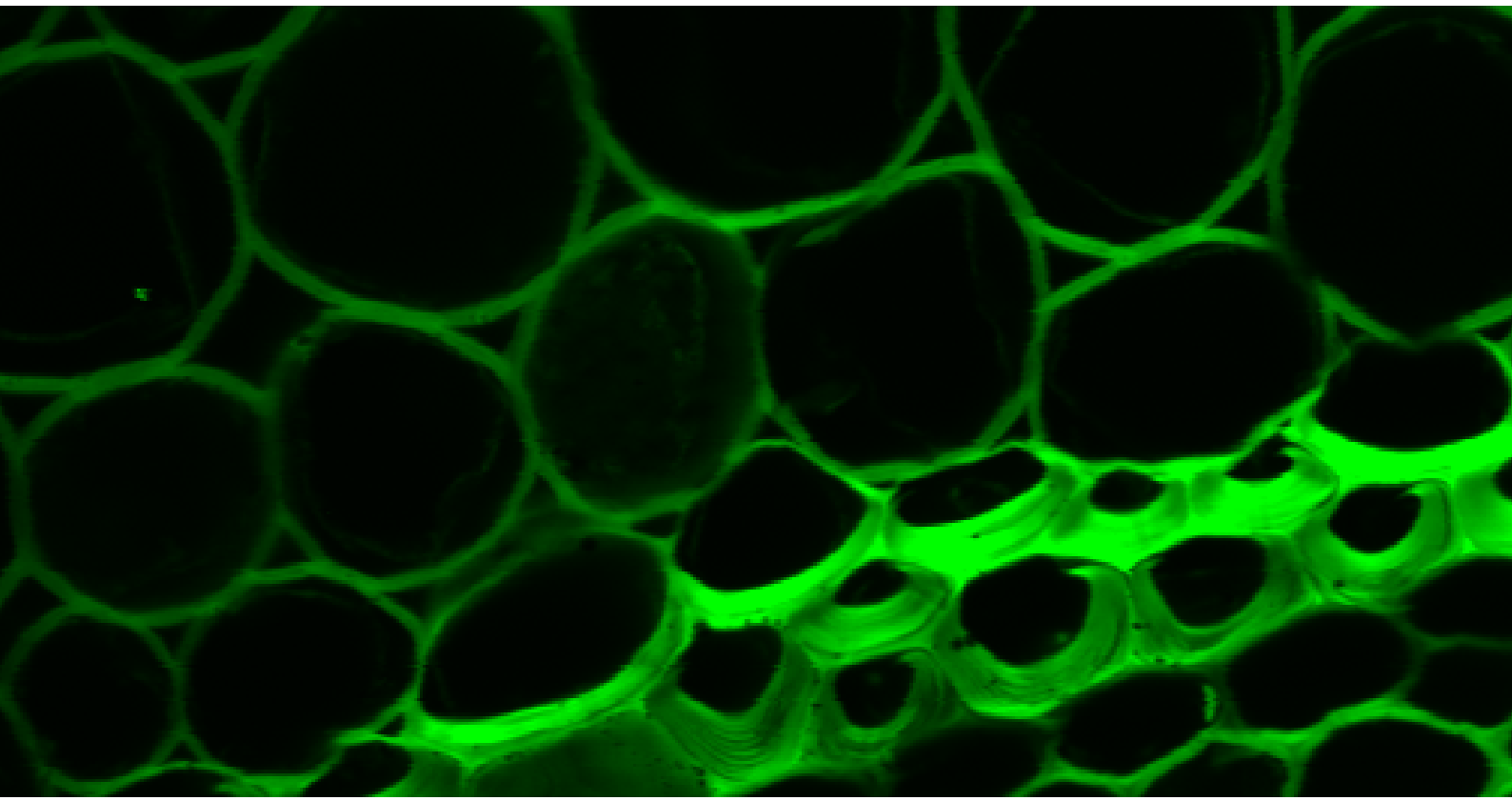
Bone remodeling, as it occurs during growth, fracture healing, but also on a daily basis, alters the physico-chemical composition of the BMM. We have shown that distinct changes of this composition influence leukaemia progression with the most profound changes close to the bone lining. Exciting results have shown that certain drugs which fine-tune the chemical milieu also heavily influence leukaemia stem cells.

All three projects have led to the discovery of innovative ways of targeting the BMM, which are to be tested in clinical trials in future.

In a fourth project we are investigating the role of lipid raft associated molecules for adhesion of leukaemia cells to the BMM. We have found that lipid raft-associated molecules play a prominent role for the engraftment of leukaemia cells, possibly via association with certain adhesion molecules.

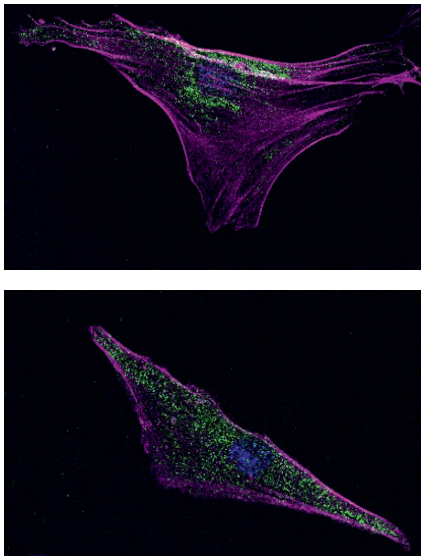
Given the current pandemic due to SARS-CoV-2 we have also initiated a projected entitled "Genetic determinants in the clinical course of infection with SARS-CoV-2" in collaboration with the German Red Cross and the Departments of Infectious Diseases, Virology and Bioinformatics at the Goethe University, 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.

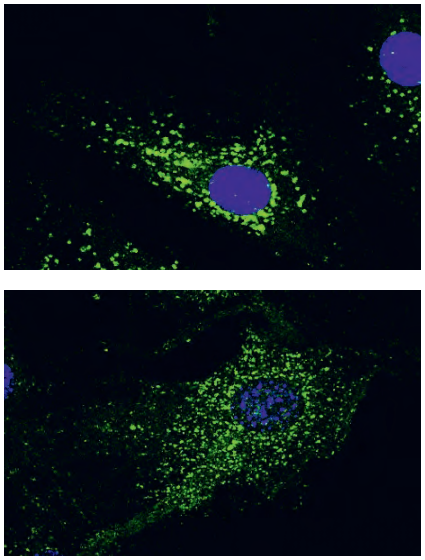


Other activities

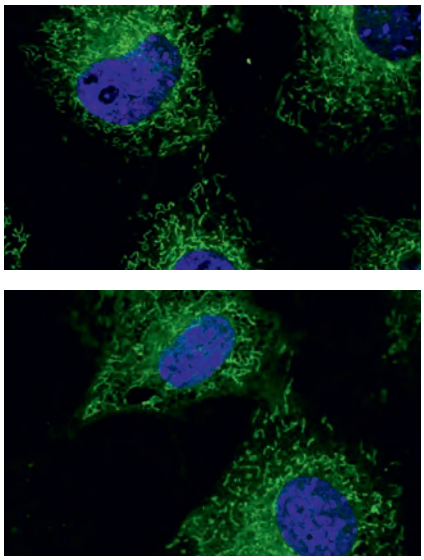
We are currently coorganizing the fourth international scientific workshop on the "Tumor environment in haematological malignancies and its therapeutic targeting" under the umbrella of the European School of Haematology to be held in London in 2021. We are also coorganizing the 22nd international scientific 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.



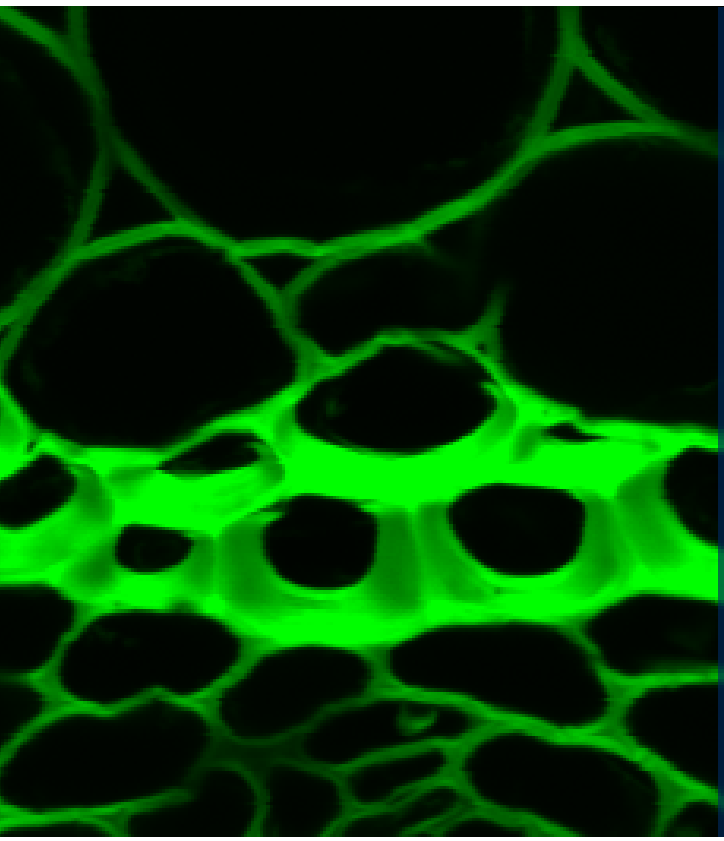
Mesenchymal stromal cells stained with DAPI (blue), syndecan 1 (green) and phalloidin (pink)



Mesenchymal stromal cells stained with DAPI (blue) and CD63 (green)



Fluorescence imaging of mitochondria using MitoTracker (dye) in mesenchymal stromal cells. The nuclei are stained with DAPI.



Ausgewählte Publikationen

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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Godavarthy PS, Kumar R, Herkt SC, Pereira RS, Hayduk N, Weissenberger ES, Aggoune D, Manavski Y, Lucas T, Pan K-T, Voutsinas JM, Wu Q, Müller MC, Saussele S, Oellerich T, Oehler VG, Lausen J, Krause DS. *The vascular bone marrow niche influences outcome in chronic myeloid leukemia via the E-selectin - SCL/TAL1 - CD44 axis. Haematologica. 2020 Jan; 105(1): 136-147*

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Verma D, Kumar R, Pereira RS, Karantanou C, Zanetti C, Minciacchi VR, Fulzele K, Kunst K, Hoelper S, Zia-Chahabi S, Jabagi M-J, Emmerich J, Dray-Spira R, Kuhlee F, Hackmann K, Schroeck E, Wenzel P, Müller S, Filmann N, Fontenay M, Divieti-Pajevic P, Krause DS. *Vitamin K-antagonism impairs the bone marrow microenvironment and hematopoiesis. Blood. 2019, 134(3): 227-238*

*co-first authorship

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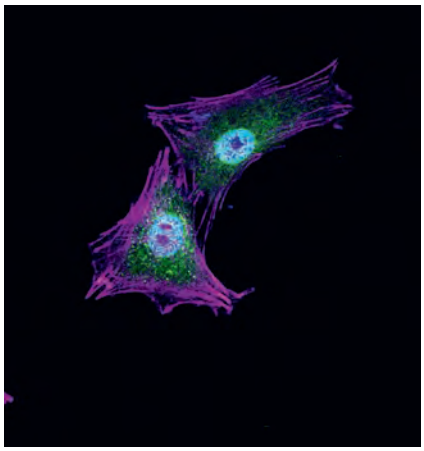
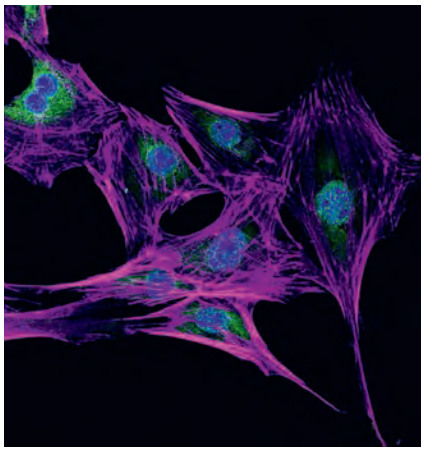
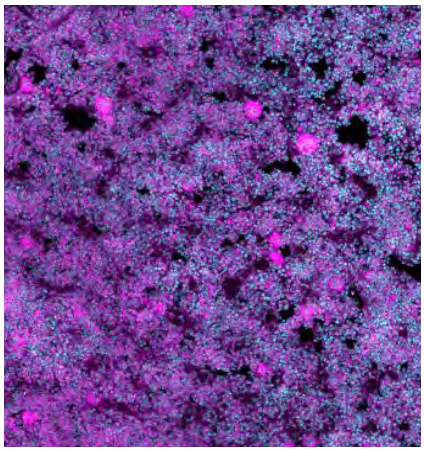
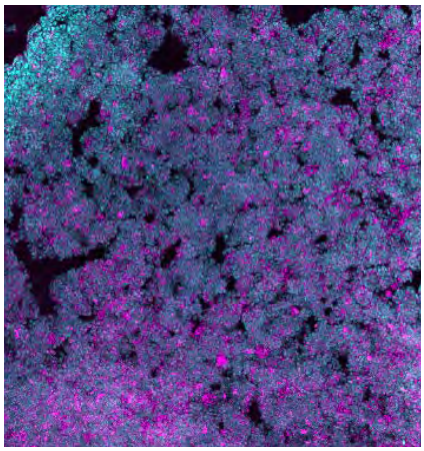


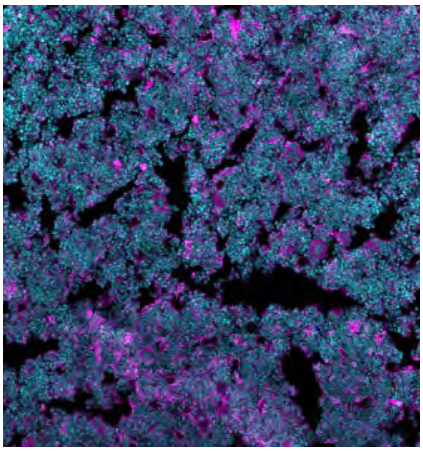


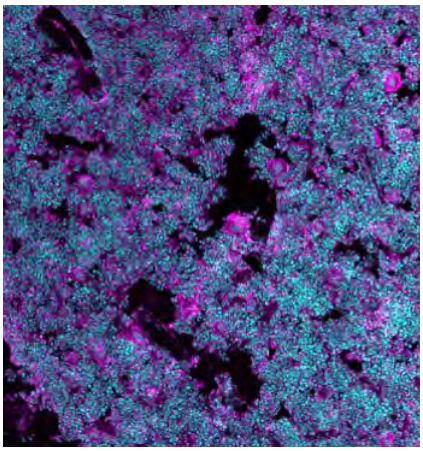
Immunofluorescence image (green) of Fatty acid binding protein 4 (FABP4) in mesenchymal stromal cell (MSC). Nuclei are stained with DAPI and the F-actin network is stained with Phalloidin.





BMP1 staining of bone sections





TGFbeta staining of bone sections

Die Knochenmarks-Mikroumgebung in Krebserkrankungen

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The Bone Marrow Microenvironment in Human Cancers

Bone marrow microenvironment

Leukemia/Metastasis

Immune escape

Although cellular transformation and cancer progression are thought to be driven by somatic mutational events that progressively provide neoplastic cells with a fitness advantage over normal cells, it is now well-recognized that the surrounding microenvironment actively contributes to this multistep process. The bone marrow represents a nurturing site for many types of blood cancers but also for disseminated bone metastatic cells which are frequently seen in some solid cancers (breast, lung and pancreatic cancers). The bone marrow microenvironment is a complex ecosystem composed of many cell types such as mesenchymal stem and progenitor cells, endothelial cells, nerve fibers as well as cells of the immune system, all of which have been shown to modulate the biology of normal hematopoietic stem cells but also that of their malignant counterpart, the so-called leukemic stem cells. Several studies, including from our group, have demonstrated that this so called "tumor microenvironment" (TME) is profoundly altered in many aspects during the tumorigenic process, including cellular composition, molecular features and functional properties. Changes in the TME also lead to important tissue and extracellular matrix (ECM) remodeling that may act in concert to promote the nurturing functions of the tumor stroma.

In this context, our lab is actively exploring the specific contributions of the bone marrow microenvironment in promoting hematological cancers, with a particular emphasis on its potential contribution to leukemia immune evasion and its impact on cellular dynamics and clonal competition in pre-leukemic syndromes, referred to as myelodysplastic syndromes. Building on the knowledge gained in the context of leukemia, we expanded our research program to explore the interplay between the bone marrow microenvironment and disseminated tumor cells from solid cancer entities, particularly breast cancer (SPP 2084, µBone). Our research program is based on the rational that in order to devise better therapeutic strategies for patients and improve outcome, we need to gain deeper insight into how malignant cells co-opt their environment to promote tumor growth.

Das Mikromilieu des Knochenmarks stellt ein komplexes Ökosystem dar in dem eine Vielzahl unterschiedlicher Zelltypen wichtige Aufgaben in der Aufrechterhaltung der Hämatopoese spielen. Allerdings können diese Zelltypen krankheitsbedingte Veränderungen aufweisen oder physiologische Funktionen werden durch Tumorzellen zweckentfremdet, um deren Entstehung und Ausbreitung zu fördern *(Medyouf, CSC, 2014, Medyouf, Blood, 2017)*. Das Forschungsziel unserer Arbeitsgruppe besteht in der funktionellen Analyse des Einflusses der Gewebsumgebung des Knochenmarks auf das Verhalten von Tumorzellen im Kontext von Blutkrebs sowie Metastasen solider Tumorarten. Unsere Arbeit stützt sich hierbei auf die Hypothese, dass die Aufklärung der Mechanismen, durch die die Knochmarks-Mikroumgebung das Verhalten der Tumorzellen beeinflusst, einen entscheidenden Beitrag zur Identifizierung neuartiger therapeutischer Ziele innerhalb der Knochenmarksnische beiträgt und Grundlagen zur Entwicklung verbesserter Behandlungsmöglichkeiten schafft.

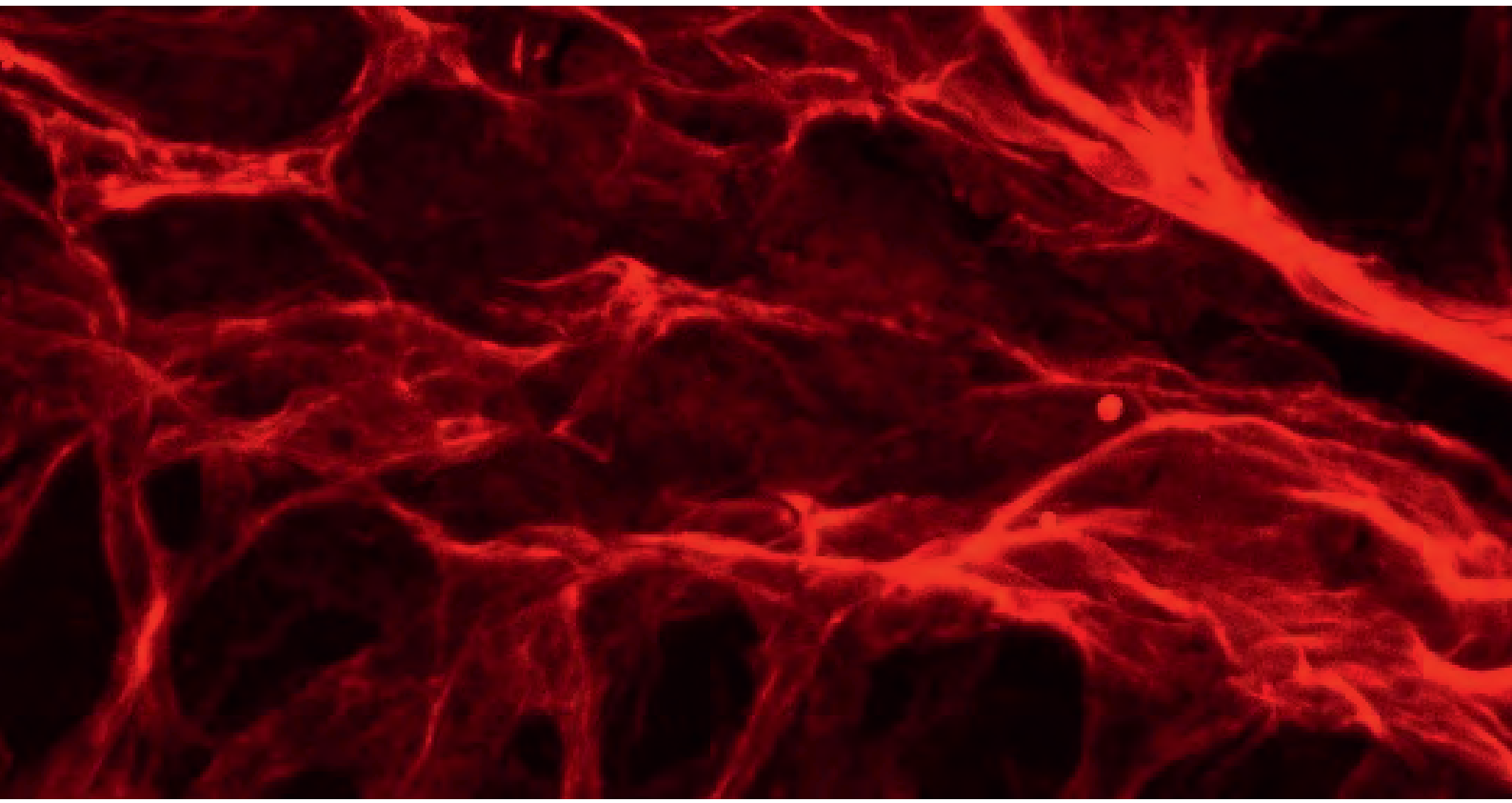
Wir verwenden unterschiedliche „Omic"-Methoden und Patientenmaterial, um die wechselseitigen Interaktionen zwischen Tumorzellen und Nischzellen zu analysieren. Ein besonderer Fokus wird hierbei auf die Rolle von Endothel-, Mesenchym- und Immunzellen gesetzt. Funktionelle und translationale Studien werden an einem breiten Spektrum an experimentellen System durchgeführt. Diese umfassen wie z.B. syngene Mausmodelle, xenotransplantierte Modelle ausgehend von Patientenmaterial (Tirado-Gonzalez, Leukemia, 2018) und humanisierte 2D und 3D Modelle der Knochenmarksnische, die kürzlich in unserem Labor etabliert wurden *(Schäffer et al., unpublished)*. Wir sind davon überzeugt, dass Therapien, die gezielt gegen Funktionen der Tumormikroumgebung gerichtet sind, einen wichtigen Teil der Bemühungen zur Etablierung verbesserter Therapieansätze darstellen und uns dem Ziel der Krebsprävention oder gar Heilung näherbringen.

Mesenchymal niche contributions to human myelodysplasia

Myelodysplastic syndromes (MDS) are heterogeneous clonal hematopoietic stem cell diseases mainly affecting the elderly and characterized by ineffective production of mature blood cells with peripheral cytopenia and the propensity to evolve to acute myeloid leukemia. Most MDS patients rely on continuous blood transfusions resulting in secondary effects leading to complications and patient deaths. The only potential curative treatment for MDS is hematopoietic cell (HCT) transplantation, which is limited to younger patients with suitable donors (<10% of MDS patients).
Our previous work revealed that patient-derived mesenchymal niche cells are essential to propagate human MDS stem cells in vivo and that human MDS cells shape their environment into a self-reinforcing one, thus highlighting the crucial role of the niche in human MDS. Ongoing work is now focusing on deciphering the interplay between hematopoietic and mesenchymal niche cells in human MDS and assessing innovative means by which we could target diseased cells to improve MDS patient outcomes. This work program includes a comprehensive molecular characterization of purified mesenchymal niche cells from primary patient material, to define new prognostic/therapeutic niche factors in MDS. The project is carried out in close collaboration with clinical partners and is generously supported by funds from the European Research Council and the German José Carreras Leukemia Foundation.
To functionally interrogate candidate factors and signaling axis that emerge from our screening efforts, we endeavor to develop disease model systems that allow us to study human cells in a relevant environment. For in-vivo studies, we generate genetically engineered mouse models (GEMM) in a highly immune-comprised background to functionally probe the function of specific niche elements and niche-produced factors in the pathogenesis of human cancers, including MDS. To reduce (3R) the number of animals used in experiments, we also develop fully humanized 2D and 3D bone marrow niche models, that can be used as higher throughput platforms to carry out functional experiments ex-vivo and are amenable to experimental manipulation such as CRISPR/Cas9 editing or ex-vivo drug screening (Fig. 1).
We are hopeful that these systems will help us translate our findings into groundbreaking novel therapeutic strategies for MDS patients, by disrupting essential niche/MDS stem cell interactions.

The immune microenvironment in acute leukemia

Acute leukemia is a group of disseminated hematological cancers that is the leading cause of cancer related-deaths in children and represents an appalling clinical challenge in adults and elderly patients. In Europe 40,000 new cases of acute leukemia are diagnosed every year. Treatment strategies are largely based on intensive chemotherapy combined with targeted therapy in specific disease subtypes (e.g. BCR-ABL inhibitors in BCR-ABL+ B-cell acute lymphoblastic leukemia), but resistance remains a leading cause of death. Although new immunotherapeutic modalities have raised hope for a subset of acute leukemia patients, both cell intrinsic (e.g. antigen loss, secondary lesions, etc)



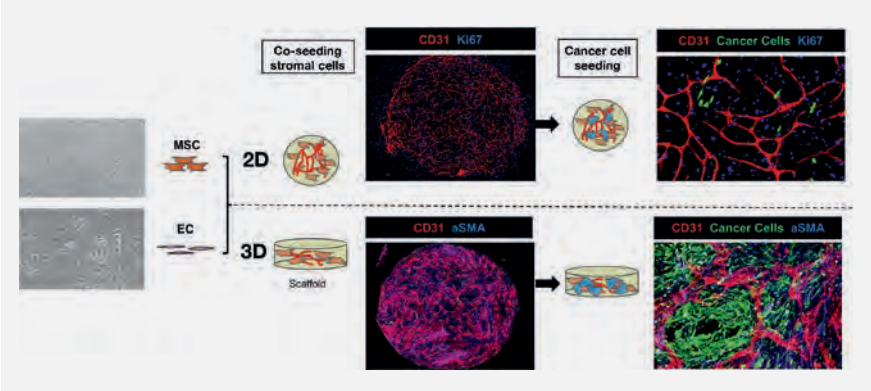
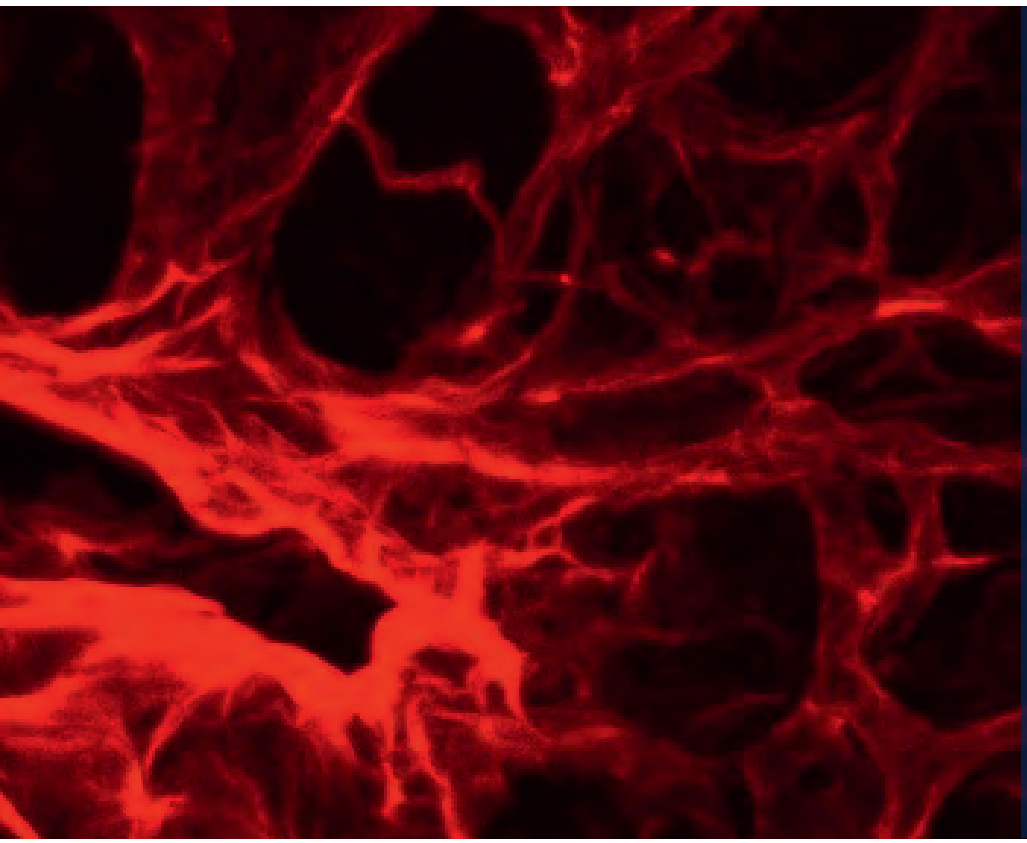


Figure 1.
Fully humanized 2D & 3D niche models. Schematic view of the 2D and 3D co-culture setting combining human derived mesenchymal stromal cells (MSCs) and endothelial cells (ECs) from bone marrow biopsies. Human CD31 marks endothelial cells and the vessel like structures that can be observed, aSMA marks activated mesenchymal cells, DAPI marks nuclei and Ki67 marks proliferating cells. Cancer cells are marked in green.

and extrinsic events (e.g. Immune suppressive microenvironment) are thought to drive treatment failure and resistance. In this context, our group is exploring the molecular mechanisms underlying the immune-suppressive functions of the microenvironment in acute leukemias and evaluating new therapeutic means by which we could improve both the immune sensing and the effector functions of the endogenous immune system, against leukemia. Our goal is to uncover new means by which we could relieve immune-suppression and trigger an effective host-versus-leukemia effect that could eliminate malignant cells. Such approaches may be particularly suited in combination with reduced intensity treatments used for older patients unfit to receive intensive regimen or allogeneic hematopoietic cell transplant, or those that exhibit minimal residual disease following induction chemotherapy. This aspect of our work is based on the analysis of patient-derived material as well as in vivo functional experiments using several genetically engineered mouse models and pre-clinical models of acute myeloid and lymphoid leukemia (Fig. 2) as well as the use of pharmacological inhibitors, some of which are currently in clinical evaluation.

Functional relevance of the extracellular matrix in the bone marrow niche

Besides the cellular environment, emerging clinical data suggest that modifications in the composition of the extracellular matrix (ECM) play a critical role in malignant progression.
In this context, our laboratory has been exploring the role of a highly conserved and multi-faceted matricellular protein, SPARC, in modulating the behavior of leukemia and metastatic bone lesions. SPARC has pleiotropic activities, such as modulation of ECM structural organization and stiffness, cellular adhesion, growth factor activity as well as other biological aspects that could impact cancer progression. Because SPARC modulates many aspects of cell-cell and cell-matrix interactions in the bone marrow, we believe that changes in SPARC expression in the TME may greatly influence the pathogenesis of hemato-



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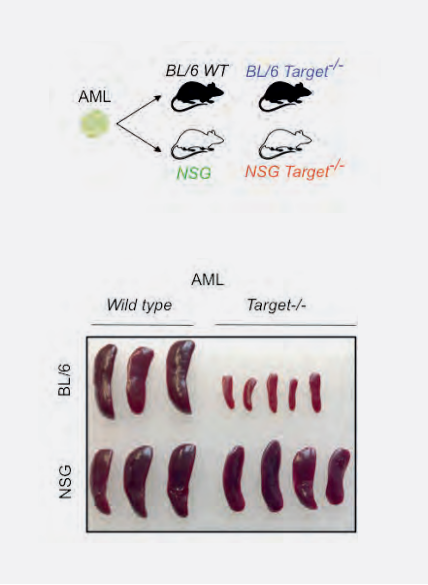

Figure 2.
Targeting a key niche-suppressive signal triggers a potent host-versus-leukemia immune response.
AML cells were intravenously injected to immune competent (C57BL/6) or immune compromised (NSG) that are either wild type or genetically modified to lack the expression of a candidate target that we recently identified as a potential immune suppressive factor in acute leukemia. C57BL/6 mice that are knock out for our identified target (BL6 Target-/-) show enhanced protection from leukemia as depicted by normal spleen size. No protection is seen in NSG counterpart (compare NSG vs NSG target-/-), indicating that the protective effects seen in BL6 Target-/- mice are strictly immune mediated.

logical cancers and behavior of disseminated tumor cells that home to the bone marrow. In leukemic contexts, cell intrinsic expression of SPARC has been shown to exert opposing effects, depending on the leukemia type or even the specific subtype under study. However, despite its high stromal expression, little is known about the specific role of niche-produced SPARC in the TME and how it impacts the biology of leukemic cells and disseminated tumor cells. Initial studies from our lab have shown that niche produced-SPARC contributes to the overall architecture of the bone marrow niche (Fig. 3), significantly impacts leukemic expansion in vivo (Tirado-Gonzalez, Czlonka E, Leukemia, 2018) and is an important modulator of metastatic dormancy in solid cancers that home to the bone marrow (Schäffer et al, unpublished). Understanding the molecular mechanisms involved downstream of SPARC may generate new insights to help devise new therapeutic strategies that could be exploited to sensitize malignant cells to current treatments.

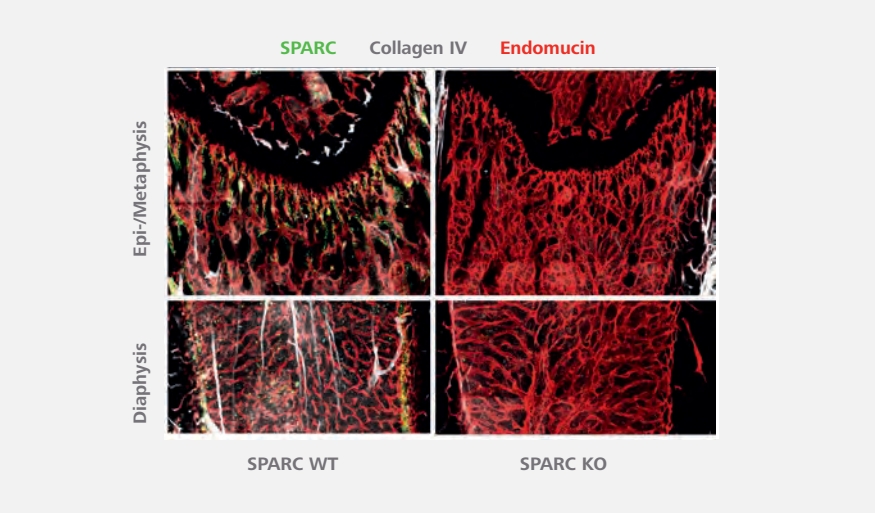

Figure 3.


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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 in cancer

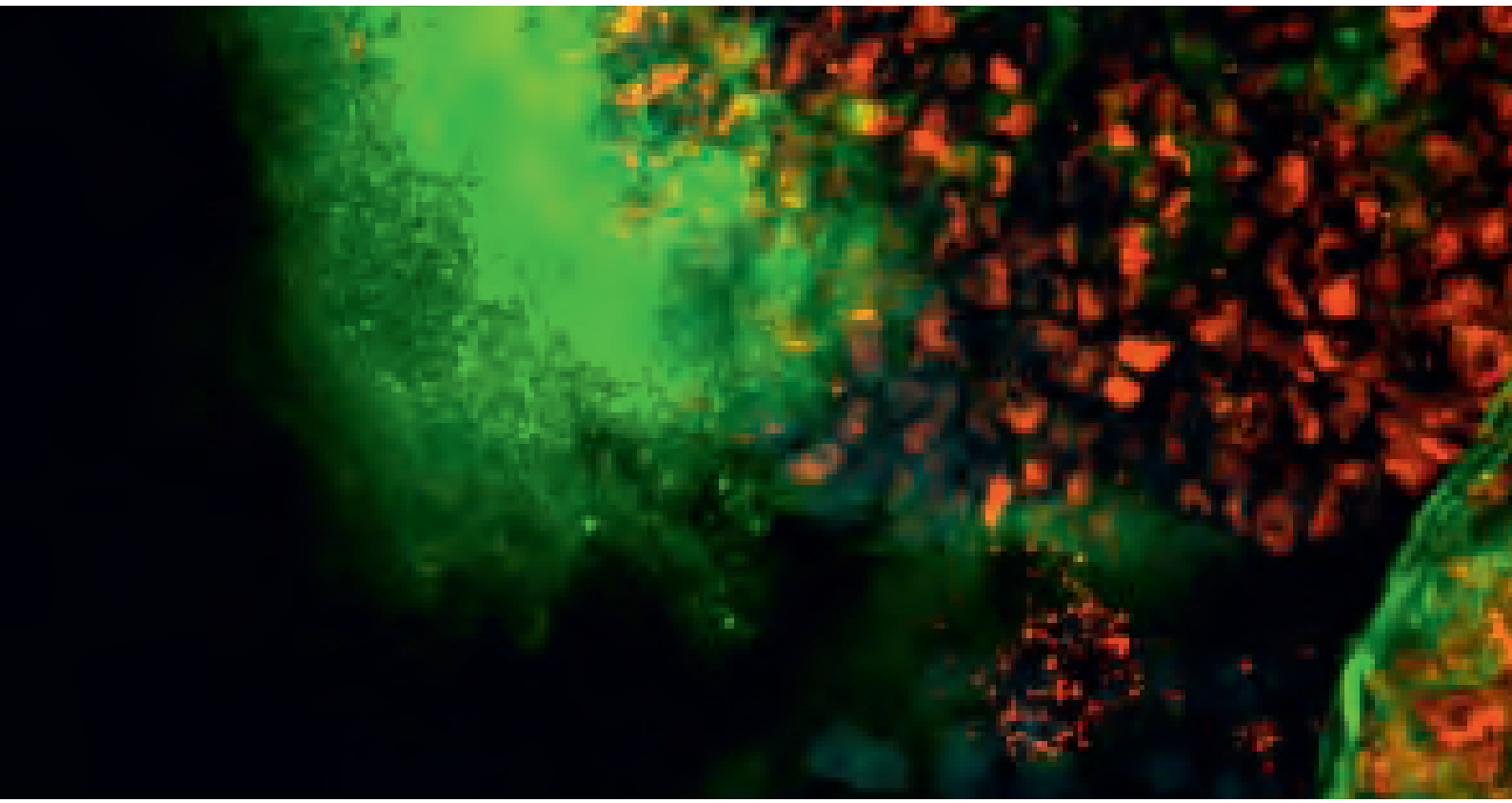
Early imaging in cancer

Diet, microbiota, and cancer

Diet is shaped by multiple diverse factors such as culture, nutritional knowledge, price, availability, taste, and convenience. Given the reciprocal interaction between host and environmental factors during carcinogenesis, food consumption is becoming critical. Due to the distinct shifts in agriculture and changes in crops in the last decades, food may have a pivotal role in aggravating disease. Our research aims at delineating how changing diet is associated with cancer initiation and progression in the pancreas and intestine at a molecular and cellular level. Using preclinical models as well as clinical samples, we aim at defining derangements in host, microbial, and tumor energy metabolism in order to define whether there are vulnerabilities that can be targeted during disease or therapy and if customizing diet may eventually pave the way for individual-based interventions.

Die Ernährung wird von vielen verschiedenen Faktoren wie Kultur, Ernährungswissen, Preis, Verfügbarkeit, Geschmack und Bequemlichkeit geprägt. Angesichts der Wechselwirkungen zwischen Wirts- und Umweltfaktoren während der Kanzerogenese, wird der Nahrungsmittelkonsum immer kritischer. Aufgrund der deutlichen Verschiebungen in der Landwirtschaft und der Veränderungen bei den Nutzpflanzen in den letzten Jahrzehnten könnte der Nahrungsmittelkonsum eine entscheidende Rolle bei der Verschlimmerung von Krankheiten spielen. Unsere Forschung zielt darauf ab, auf molekularer und zellulärer Ebene zu beschreiben, wie eine veränderte Ernährung mit der Krebsentstehung und dem Fortschreiten von Krebs in Bauchspeicheldrüse und Darm verbunden ist. Mit Hilfe präklinischer Modelle und klinischer Proben wollen wir Störungen im Wirts-, Mikroben- und Tumorenergiestoffwechsel definieren, um festzustellen, ob es Schwachstellen gibt, die während der Erkrankung oder Therapie gezielt angegangen werden können, und ob eine angepasste Ernährung schließlich den Weg für individuelle Interventionen ebnen kann.

Figure 1.
Using various technology platforms that we established, we are investigating alterations in metabolism during disease and therapy in clinical samples and preclinical models.



Metabolic Derangements during Cancer

Cancer is marked by dysregulation of the signaling pathways that orchestrate proliferation, cell death, tumor-promoting inflammation and energy metabolism. Our studies focus on elucidating the host and tumor energy metabolism, delineating the critical alterations that take place during disease initiation and progression, and targeting the metabolic vulnerability of tumor cells genetically or pharmacologically in relevant mouse models. This may have a diagnostic value in pancreatic and intestinal cancer and can impact therapy response. Using mouse- or human-derived organoids as a model system, we characterize the metabolic potentials, compare them to its primary tumor tissue, and eventually use them as a tool to target vulnerabilities in energy metabolism.

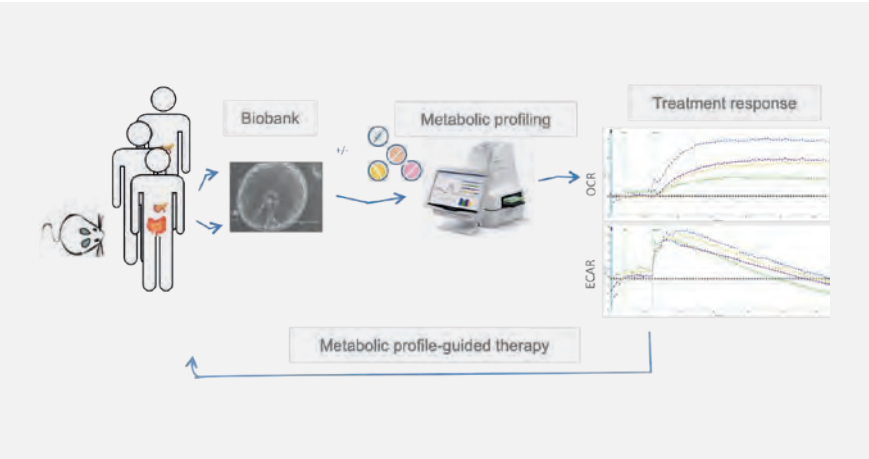
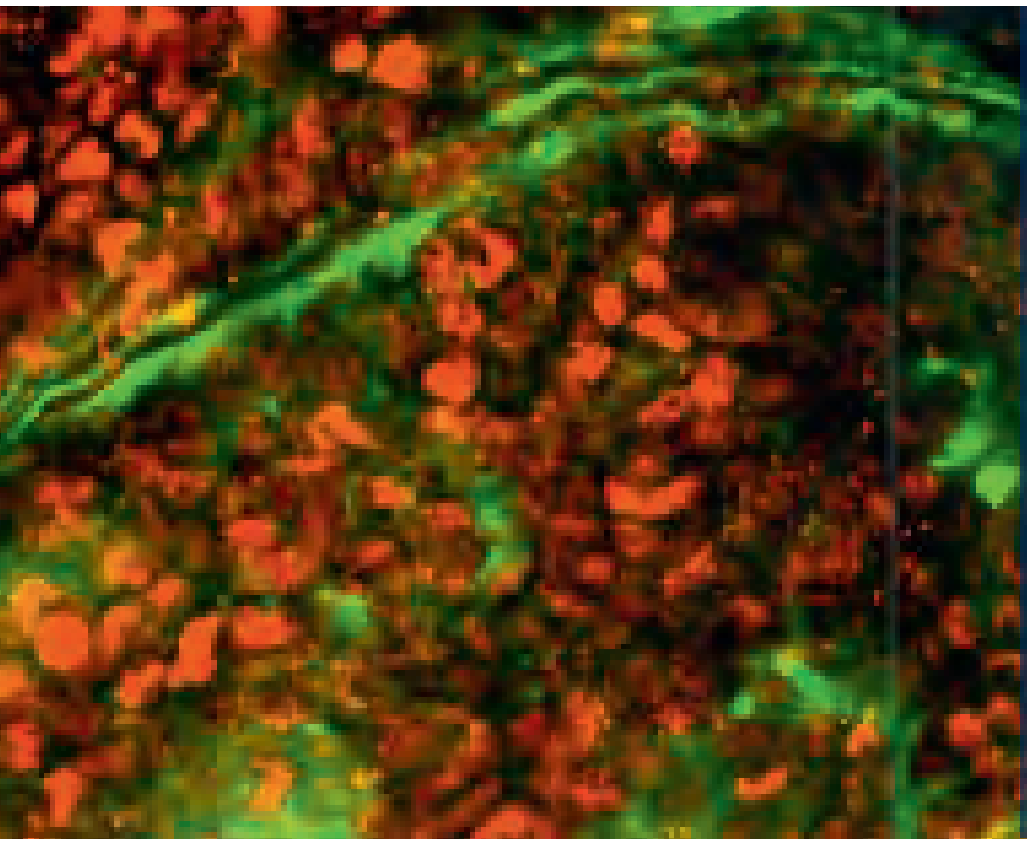


Figure 2.
Using preclinical models, derangements in bioenergetic pathways during tumor development and therapy are investigated in order to define and target vulnerabilities, which may set the stage for future drug discovery for therapeutic interventions.

Bioimaging in Cancer

Late-stage presentation, inaccessible diagnosis and resistance to therapy represent the most common causes of increased mortality in cancer patients. Although, the conventional imaging modalities are high-end technologies, they still have restrictions with respect to their resolution, sensitivity, generating contrast, high costs, and side effects, suggesting the urgent need for alternative approaches for cancer screening, diagnosis, and monitoring of treatment efficacy. Our studies aim at identifying reliable biomarkers for early detection, developing approaches that target defined changes during specific stages of tumor development and progression for bioimaging using MRI-detectable smart probes.



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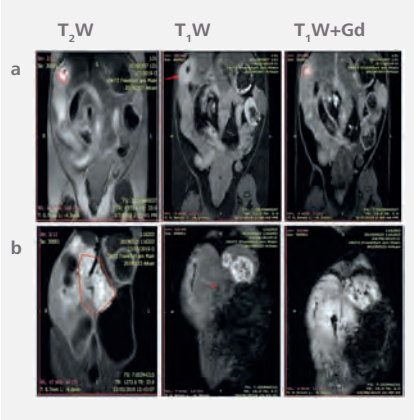
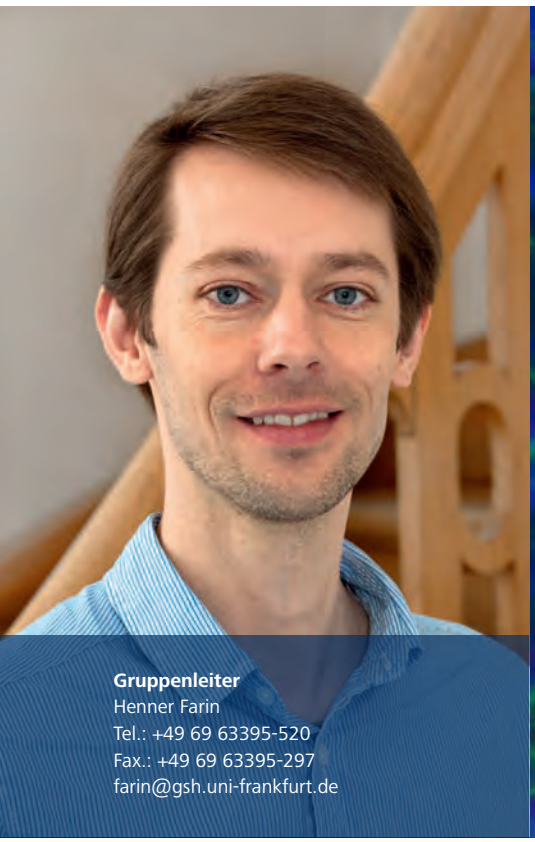


Figure 3.
Magnetic resonance imaging of tumors in preclinical mouse models. Coronal images showing a tumor located in the a. proximal duodenum, b. pancreas, which appears darker in T_1-weighted Flash 3D pre-contrast scan and becomes brighter in post-Gd, after *i.v.* injection of Gadovist, the contrast agent.

Diet, Microbiome, and Cancer

The human gut is inhabited by billions of bacteria contributing majorly to the regulation of metabolic functions and immune homeostasis. Given that microbiota composition and functional profiles shape susceptibility to cancer under deranged metabolism, the dynamics of bacterial community is critical. Nutrition can directly or indirectly modulate the microbiome and play a decisive role in disease outcome. Our studies aim at unravelling the impact of varying dietary intake 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 metabolism.

Figure 4.
Defining the alterations in microbiome and the effect of interventions designed to impact microbial community and function during disease and therapy is our ultimate goal.



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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 60,000 new diagnoses and 25,000 death cases each year. Two major classes can be distinguished: microsatellite stable (MSS, ~85%) and microsatellite instable tumors (MSI; ~15%). Although great advances have been achieved in tumor prevention, the therapeutic options for patients with advanced disease are limited. Main challenges are a high genetic heterogeneity in CRC both at the inter-individual and intratumoral level. In addition, prognosis and therapy response are strongly influenced by the tumor microenvironment. The improved molecular and cellular understanding has unfortunately not yet led to more effective therapies. One limitation is the availability of predictive cancer models to test treatment strategies. Patient-derived tumor organoids (PDTOs) have emerged as an important preclinical model for CRC. The organoid technology is based on expansion of primary epithelial cells in 3D Matrigel and defined growth factors. Originally developed for the mouse small intestine, the culture conditions have been adapted to support growth of normal and tumor cells from

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-abgeleiteten 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.

Unsere Gruppe am Georg-Speyer-Haus wird finanziert vom Deutschen Konsortium für Translationale Krebsforschung (DKTK) und Deutschen Krebsforschungszentrum (DKFZ).

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 the Frankfurt Cancer Institute, we are generating a CRC organoid biobank as a research tool to study phenotypes including drug sensitivity and therapy resistance. In addition, we develop genetic technologies for targeted modification of primary 3D organoids.

The Farin group is funded by the German Cancer Consortium (DKTK) and the German Cancer Research Center (DKFZ) at the Georg-Speyer-Haus Frankfurt. Research focus areas are:

I. Maintenance and differentiation of intestinal stem cells during homeostasis

Tissue homeostasis and regeneration depends on the capacity of stem cells to proliferate and to produce differentiated offspring. In the past years it has been recognized that signals from the 'stem cell niche' govern turnover and plasticity of stem cells to meet the physiological demands (Tetteh/Farin/Clevers Trends Cell Biol 2015). Organoids can serve as accessible models to investigate the mechanisms that govern homeostasis. We could identify that the Wnt3 protein is secreted by niche cells and specifies stem cells in close vicinity (Fig. 1; Farin et al., Nature 2016). Our findings suggest that localized production and the limited mobility of Wnt3 regulate the self-organization of the intestinal epithelium into proliferating crypts and differentiated villus compartments. In addition, we have analyzed intestinal epithelial organoids as a model for microvillus inclusion disease, a human congenital defect that affects intracellular vesicle trafficking. Confocal time-lapse microscopy in reporter-transgenic organoids revealed that the cellular phenotype is associated with the level of cell differentiation (Mosa et al., Cellular and Molecular Gastroenterology and Hepatology 2018).

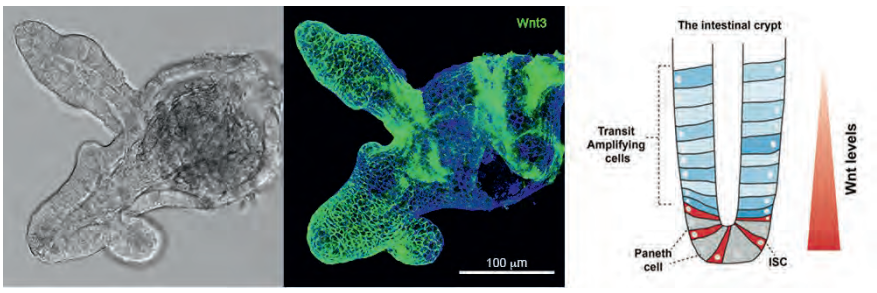
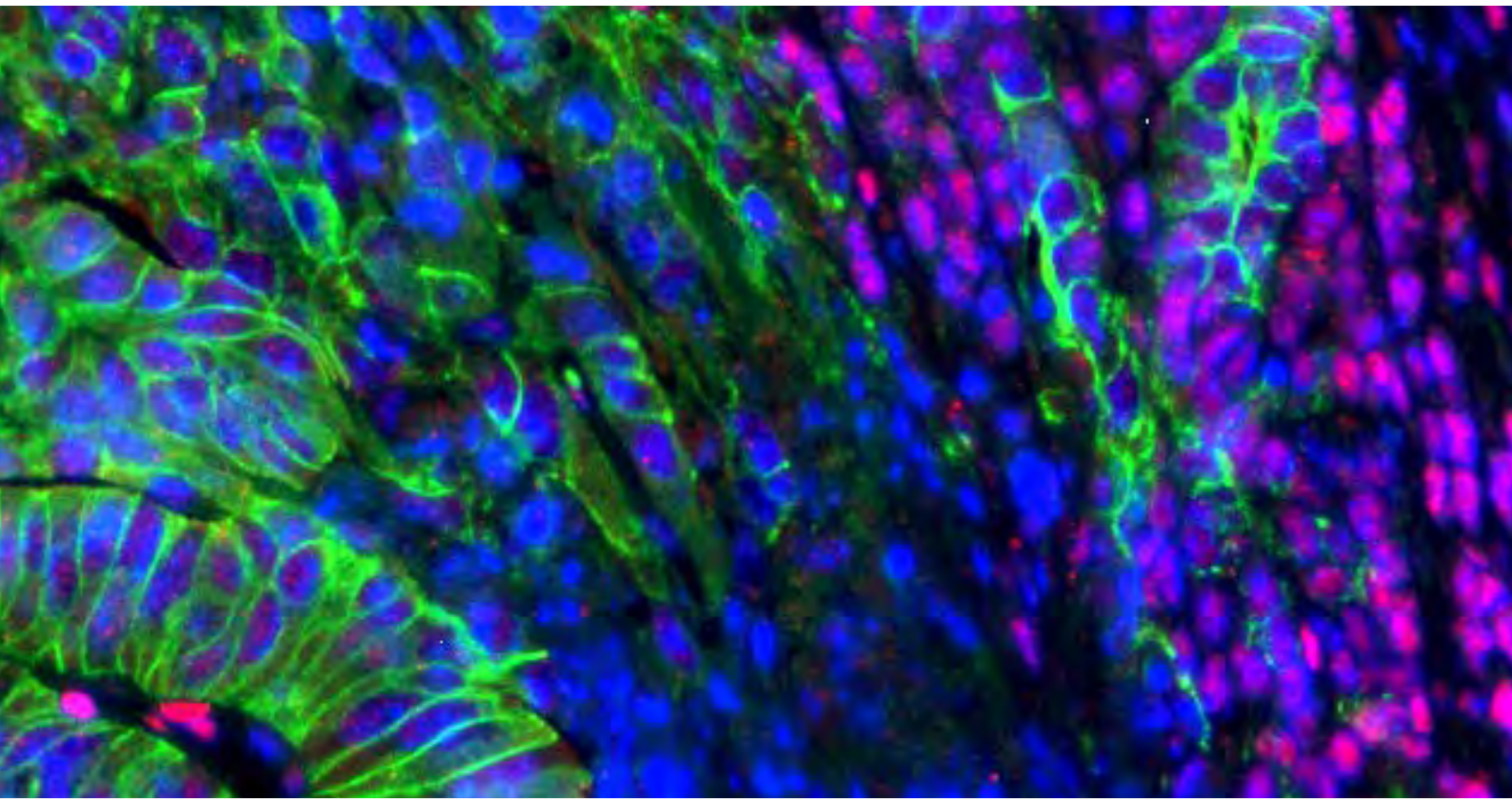


Figure 1.
Study of Wnt signals in self-renewal and differentiation.
Localized production of Wnt3 in mouse intestinal organoids (left, refer Farin et al., *Nature* 2016). The resulting Wnt gradient patterns the epithelium into zones of stem cells and differentiated cells (right, from commentary of Gregorieff and Wrana, *Cell Research* 2016).



II. Functional genetic screening to identify new CRC driver mutations

Oncogenes and tumor suppressors show context-specificity that depends on the tumor type, the cell of origin, the genomic background and environmental factors. The genetic heterogeneity in CRC creates a demand for patient-specific experimental models. We have previously studied the transcriptomic and proteomic changes induced by known oncogenic mutations in CRISPR/Cas9-engineered organoids (Michels et al., J. Exp. Med. 2019). Genetic screening can also provide information about previously unknown cancer driver mutations. However, this technology has not been available for 3D cell models. To fill this gap, we have devised a platform for pooled CRISPR/Cas9 screening in human colon organoids (Fig. 2). We use custom generated gRNA libraries to identify tumor suppressors in vitro and after organoid xenotransplantation. Furthermore, we have combined our library with unique molecular identifiers (UMIs) to study the consequences of gene perturbation on the clonal level, representing a powerful method for phenotypic characterization in heterogeneous tumors (Michels et al., Cell Stem Cell 2020).

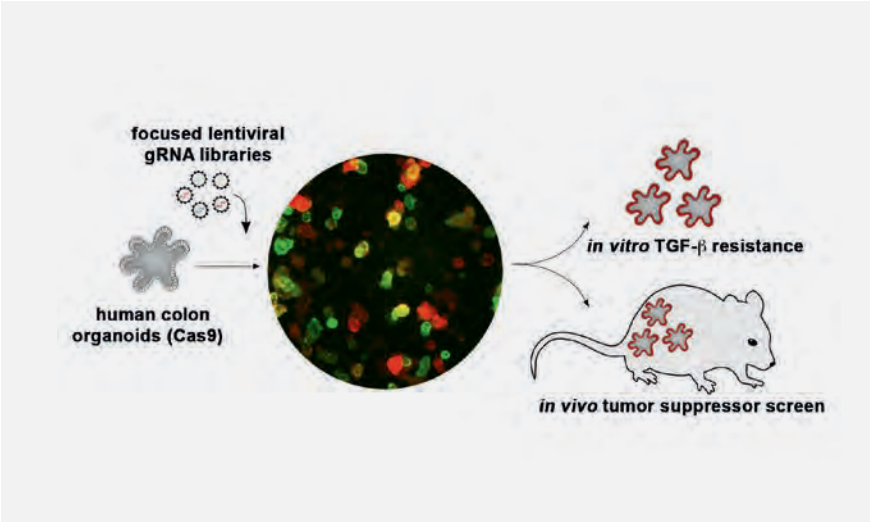
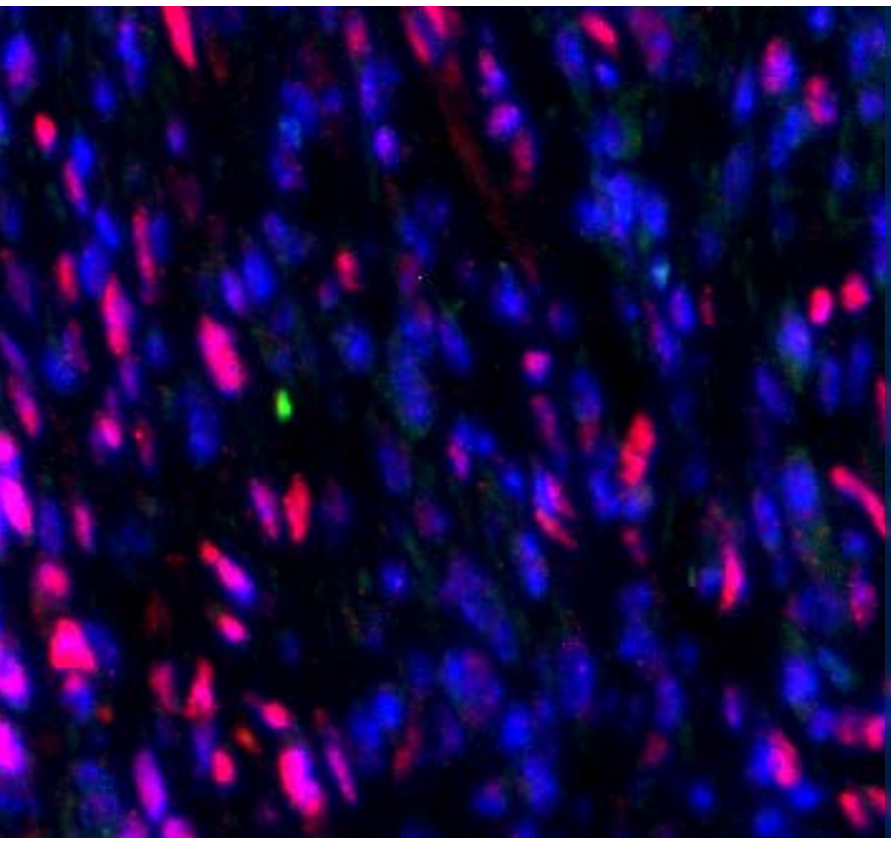


Figure 2.
Pooled CRISPR-Cas9 Screening in Human Colon Organoids
Graphical abstract adapted from Michels et al., *Cell Stem Cell* 2020. The central image shows human colon organoids after stochastic transduction with GFP- and RFP-containing lentivirus.



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*co-correspondence

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Schnalzger TE, de Groot MHP, Zhang C, Mosa MH, Michels BE, Röder J, Darvishi T, Wels WS, Farin HF. (2019)
3D model for CAR-mediated cytotoxicity using patient-derived colorectal cancer organoids. ***EMBO Journal***

Michels BE, Mosa MH, Streibl BI, Zhan T, Menche C, Abou-El-Ardat K, Darvishi T, Członka E, Wagner S, Winter J, Medyouf H. Bourtros M, Farin HF. (2020)
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III. Establishment of pre-clinical organoid models for cancer immunotherapy

In CRC, the majority of MSS patients do not respond to immune checkpoint inhibitor therapy. To improve the response, lymphocytes can be engineered to specifically recognize tumor-associated antigens, however, application of such chimeric antigen receptors (CAR)-modified cells is 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 PDTO co-culture model to test CAR-cells (Fig. 3; Schnalzger et al., EMBO Journal 2019). In collaboration with Prof. Winfried Wels (Georg-Speyer-Haus), we have used CAR-modified NK-92 cells directed against various tumor-antigens and measured tumor cell killing by an enzymatic assay. In addition, we have performed confocal live imaging to monitor effector cell recruitment and cytotoxicity at a single organoid level. Our platform may help to evaluate CAR efficacy and specificity for personalized immunooncology. As participant of the recently established EU-consortium 'EUbOPEN' ('Enabling and unlocking biology in the OPEN') organoid models will be used for pharmacologic testing. The project is funded by the 'Innovative Medicines Initiative' (IMI2) and we will develop 'Human Tissue Assays' for (immuno)-oncology in CRC and subsequently conduct high-throughput drug screens.

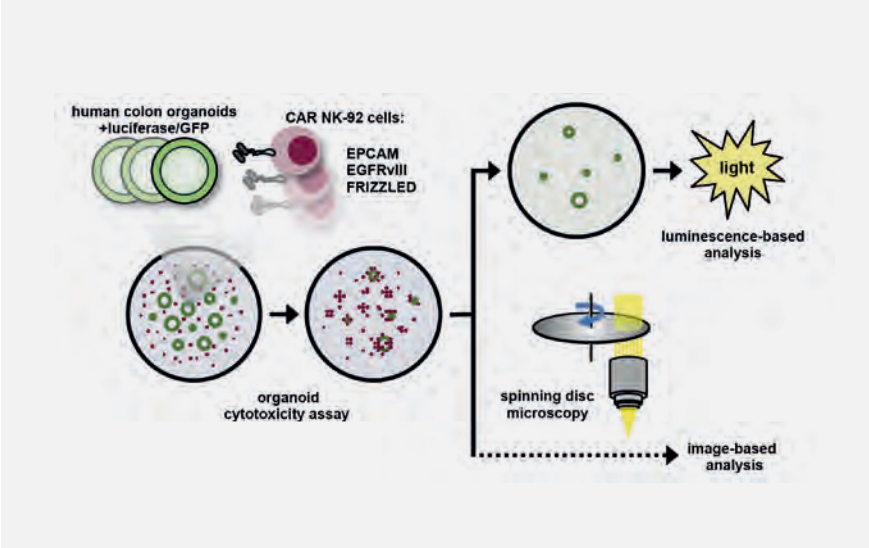

Figure 3.
3D model for CAR-mediated cytotoxicity using patient-derived CRC organoids
Graphical abstract from Schnalzger et al., *EMBO Journal* 2019.

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Zell-Zell Interaktion und Plastizität im entzündlichen Tumormikromilieu

Cell-cell Interaction and Plasticity in the tumor microenvironment

Colorectal carcinogenesis

Inflammation in tumor development, progression and therapy

Cell plasticity in the tumor microenvironment

Colorectal carcinoma (CRC) belongs to the three most common and lethal cancer entities in industrial countries. The risk of developing CRC increases with age and the majority of cases occur in people aged 50 or older. Risk factors include family history of CRC, inflammatory bowel diseases (IBD), genetic syndromes and lifestyle factors such as low-fiber and high-fat diet, obesity, tobacco use and alcohol consumption, which presumably account for the increased prevalence in "the western world". The known fact, that lifestyle factors can influence the probability of CRC development, already hints to the crucial finding that there is more to colorectal carcinogenesis than cell intrinsic oncogenic mutations, namely the tumor surrounding microenvironment. Although DNA mutations in tumor initiating cells are undoubtedly required, the microenvironment, consisting of stroma cells and their secreted chemokines and growth factors, as well as the intestinal microbiota are essential for tumor development as well and can shift the course of the disease to a favorable or poor outcome. In general, inflammatory processes are associated with various kinds of cancer, in

Der Fokus unserer Forschung liegt auf der funktionellen Analyse des Mikromilieus im Kolonkarzinom. Unter Verwendung von dreidimensionalen Zellkulturen und konditionalen Knockout-Mäusen, mit denen wir eine zelltypspezifische Aktivierung oder Inaktivierung bestimmter Gene erreichen, führen wir funktionelle Untersuchungen durch. Hierbei kommen relevante Mausmodelle für das Kolonkarzinom zum Einsatz, welche die verschiedenen Arten und Stadien der Tumorentstehung valide abbilden. Seit vielen Jahren beschäftigen wir uns mit der systematischen Analyse eines entzündlichen Mikromilieus im sporadischen und Entzündungs-assoziierten Kolonkarzinom. Obwohl inflammatorische Prozesse allgemein tumorförderlich sind, ist eine inflammatorische anti-tumor Immunantwort besonders in der Therapie ausdrücklich erwünscht. Ebenso können die Zellen des Tumorstromas durch die von ihnen sezernierten Zytokine und Wachstumsfaktoren positiv oder negativ auf die Tumorprogression einwirken. Dass ein und der selbe Zelltyp je nach Tumorentität, Mikromillieu, Patient oder einfach nur Lokalisation innerhalb eines Tumors komplett gegensätzlich wirken kann, ist ein Beispiel von Zellplastizität. Die molekularen Prozesse, die Immun- und Stromazellen auf unterschiedliche Weise polarisieren, sowie differenzierte Zellen dazu bringen zu dedifferenzieren und stammzellartigen Charakter zu erlangen, sind bis heute nicht vollständig geklärt und Kernpunkt verschiedener unserer Forschungsprojekte.

Durch die Erkenntnisse solch grundlegender Prozesse soll die Pathogenese des Kolonkarzinoms aufgeklärt werden und neue therapeutische Ansätze aufgedeckt werden um das Leben der Patienten zu verlängern und deren Lebensqualität zu verbessern.

a multitude of organs, however, colorectal cancer represents a particular good model to study the interaction between inflammation and cancer. Apart from chronic infections, autoimmune diseases or exposure to harmful substances (e.g. alcohol or drugs, correlated to hepatitis), also obesity or hyperglycemia cause low grade smoldering inflammation, that can contribute to carcinogenesis.

The tumor stroma consists of various cell types, including fibroblasts, vascular cells and recruited immune cells that communicate with each other and the tumor cells by the release of cytokines that act in a paracrine, autocrine and juxtacrine manner to activate intracellular signaling pathways, which in turn polarize the cells in a certain fashion (Figure 1). The respective polarization profile, especially of infiltrating immune cells, contributes to the high plasticity observed in tumors and whether immune cells confer pro- or anti-tumorigenic properties. Next to this functional shift, cell plasticity also refers to a profound switch of differentiated cells, either back into a more undifferentiated, stem cell like type, or in completely distinct

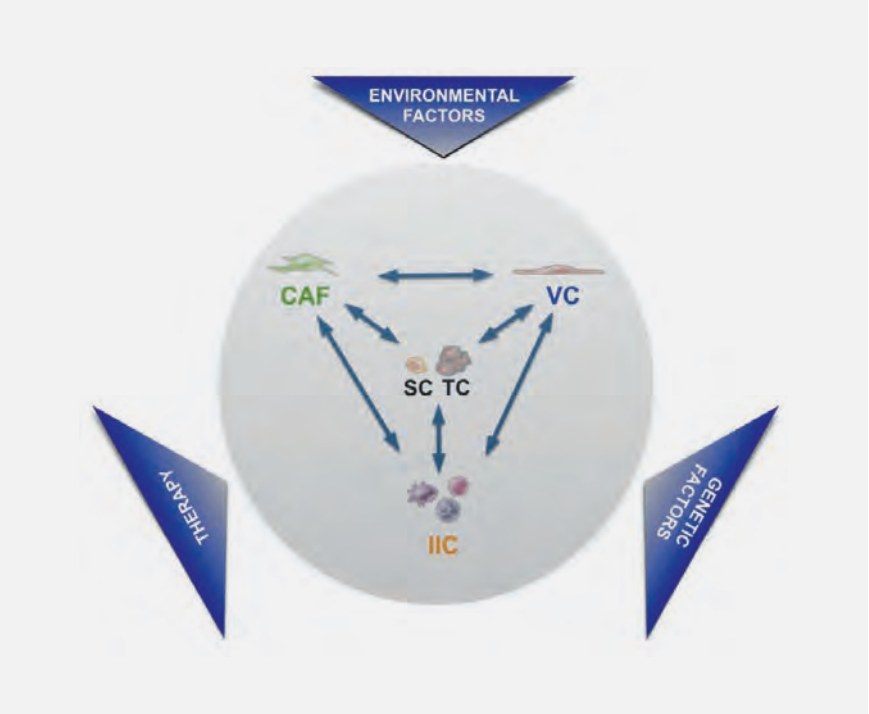
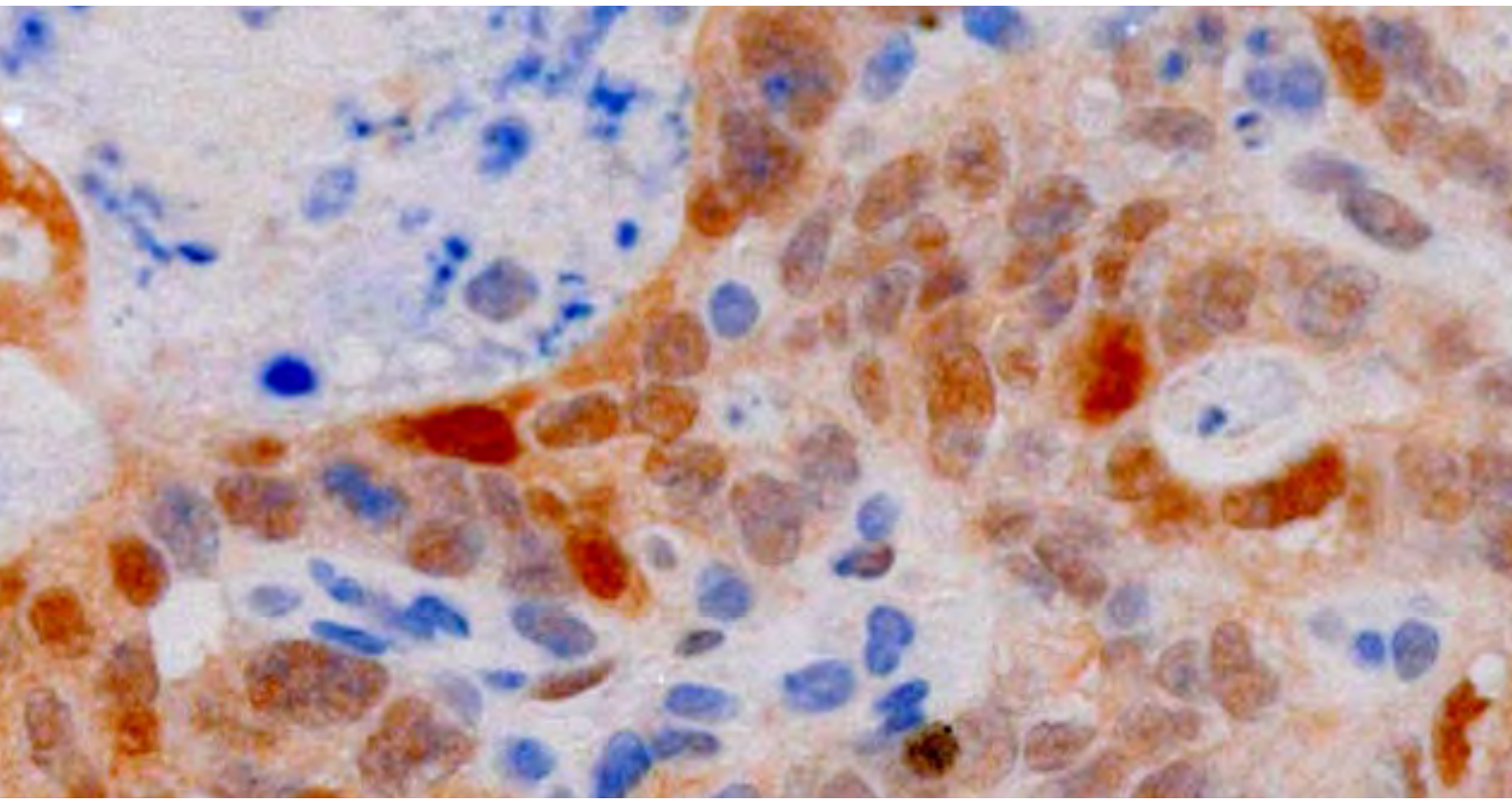
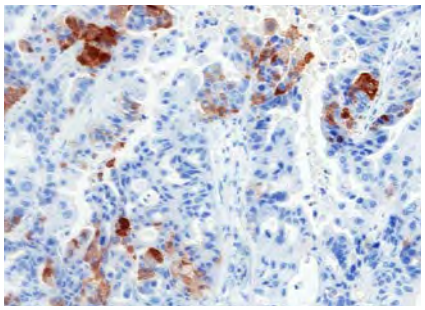


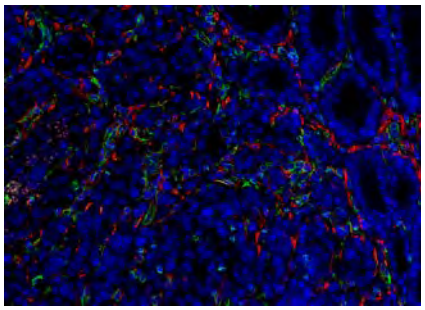
Figure 1.
Schematic representation of the dynamic crosstalk of the different cell types within the tumor. Stem cells (SC) and tumor cells (TC) are in bi-directional crosstalk with cells of the tumor stroma (CAF, cancer-associated fibroblasts; VC, vascular cells; IIC, infiltrating immune cells). Conversely, there is also significant crosstalk between the different cells of the tumor, which molecularly and functionally influence each other to exert pro- and anti-tumorigenic effector functions. Both the stem/tumor cell compartment and the stromal compartment are further influenced by environmental factors (i.e. tobacco, alcohol, diet, UV irradiation, the commensal microbiome, pathogenic bacteria and viruses) as well as genetic factors and tumor therapy (i.e. chemotherapy, irradiation).

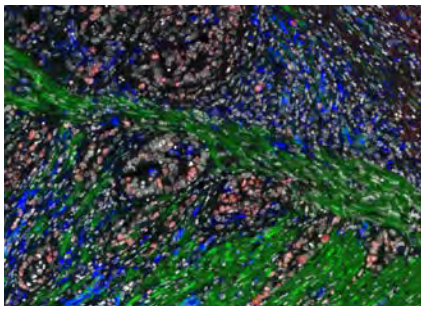


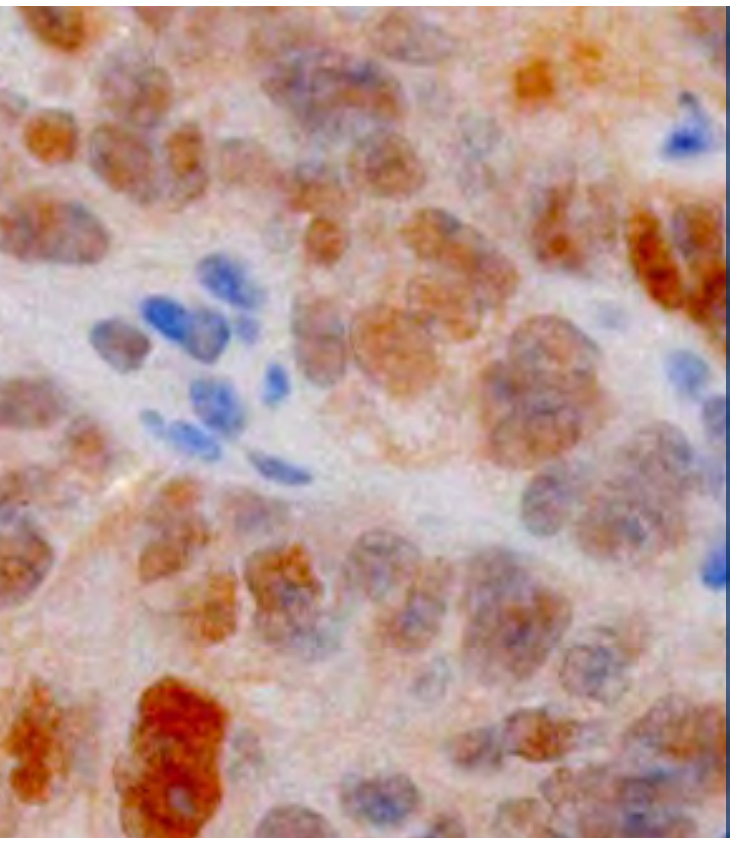
cell types, as observed in the epithelial-to-mesenchymal- or mesenchymal-to-epithelial transition (EMT or MET). EMT, an essential embryonic process, allows for metastatic spread if aberrantly activated in malignant epithelial cells, that need to undergo MET after successful seeding to metastatic sites, where they form metastases, phenotypically resembling the primary tumor. In case of CRC, crypt like structures are formed in liver or lymph nodes. The activation of this transdifferentiation programs depends on microenvironmental signals and is orchestrated by a network of transcription factors that together with epigenetic regulators control the expression of proteins involved in cell polarity, cell–cell contact, cytoskeleton structure and extracellular matrix degradation, including the repression of key epithelial or mesenchymal genes respectively. Some of the underlying molecular mechanisms and key players are well established, like EMT activating transcription factor families ZEB, TWIST and SNAIL. However, the exact signaling networks and participating cell types that are involved in the promotion of cellular plasticity have only been partially unraveled so far. Another interesting example of this depicts the recurrence of Lgr5-positive intestinal stem cells following their chemical abrogation. Several differentiated cell types of the crypt seem to gain the ability to dedifferentiate back into Lgr5-positive stem cells and repopulate the formerly depleted pool. How this can be achieved on a molecular level is one of our current research projects.

Recently, based on gene expression profiles of colorectal tumors a consensus molecular subtype (CMS) classification has been established, which impressively demonstrated that a distinct mesenchymal subtype characterized by high stromal infiltration, TGFβ1 activation and angiogenesis is associated with shorter overall and relapse-free survival, thus, highlighting the importance of the tumor microenvironment for disease progression in contrast to a particular set of mutations. A model recapitulating such tumors does not exit so far. We have examined the role of constitutive AKT activation in an AOM-induced colon cancer model based on an intestinal epithelial cell specific p53 deletion (Schwitalla, S. et al., Cancer Cell, 2013). As it turned out AOM challenged









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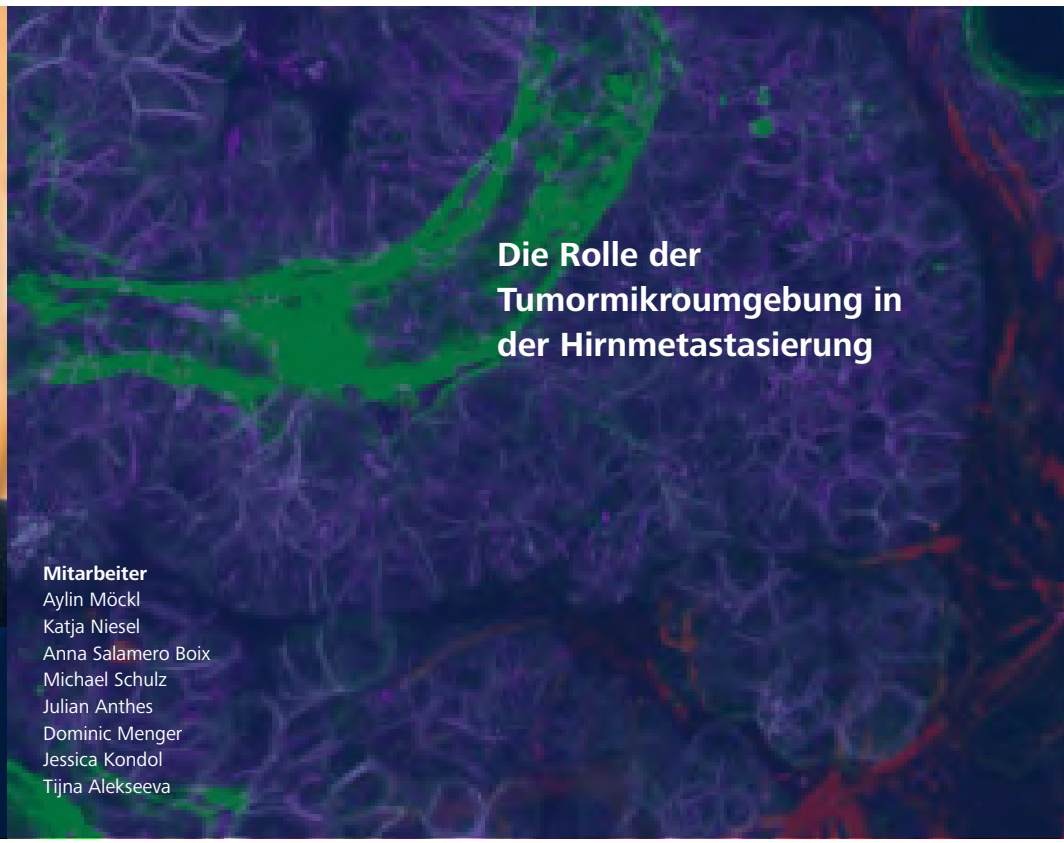
mice lacking p53 and that express at the same time a constitutively active form of AKT in IEC become more metastatic than AOM injected *Tp53*$^{\Delta IEC}$ animals. Even more importantly, the resulting colon tumors resemble human tumors of the poor prognosis subtype. We were able to identify a strong Notch activation in these tumors and found particularly upregulation of Notch3 but surprisingly not Notch1. Neutralization of Notch3 blocks metastasis and expression of a constitutively active Notch3 (Notch3IC) in the absence of mutant AKT strongly enhances metastasis in a newly developed model of orthotopically transplanted tumor organoids that does not depend on submucosal injection of organoids and therefore faithfully recapitulates all stages of invasion. Furthermore, we have carefully analyzed various human colorectal cancer cohorts and find a clear correlation between Notch3 and tumor progression, decreased patient survival and most importantly a distinct Notch3 upregulation in mesenchymal tumors. Thus, we have developed a new mouse model for a particular subtype of tumors and propose that Notch3 may represent a new target for treatment of CMS4 tumors and prevention of metastatic disease supporting the notion of an individualized therapy for CRC which does not exist so far (Varga et al., 2020).



Die Rolle der Tumormikroumgebung in der Hirnmetastasierung

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Microenvironmental regulation of brain metastasis

CNS immune landscape

Brain metastasis-associated inflammation

Radio-immunotherapy

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 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. Consequently, strategies that aim to reactivate T cell function in the CNS will be blunted by immune suppressive functions of myeloid cells. Hence, immune-modulatory strategies that transiently revoke the suppressive milieu in BrM are expected to synergize with immunotherapy. We therefore seek to gain detailed insight into the complex interplay between innate and adoptive

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. Ein besonderer Fokus liegt hierbei auf der Identifizierung von Gensignaturen tumor-assoziierter Immunzellen. Wir erhoffen uns hierdurch wichtige Erkenntnisse zur Aufklärung der Mechanismen zu gewinnen, durch die Krebszellen tumorfördernde Funktionen in Zellen der Gewebsumgebung induzieren und körpereigene Abwehrreaktionen hemmen. Unser Ziel besteht darin, dieses Wissen in wirksame Therapieansätze zu übersetzen und in präklinischen Modellen zu überprüfen.

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 neurotoxic tissue damage (Fig.1).

Functional dichotomy of brain-resident microglia and monocyte-derived macrophages in brain metastases

Tumor-associated macrophages (TAMs) are the most abundant non-cancerous cell type in brain metastases constituting approximately 30% of the tumor mass. The origin of TAMs in primary and metastatic brain tumors has long been controversially discussed given the difficulties in discriminating brain-resident microglia from recruited monocyte-derived macrophages in the context of brain malignancies. Technological and conceptual advances now provide unprecedented opportunities to study brain resident and recruited immune cells in brain metatases and evaluate common or lineage-specific functions in disease progression and therapy response. Using a comprehensive set of brain metastasis models, we found that the majority of TAMs in brain metastases originate from yolk sac-derived brain resident microglia (TAM-MG), whereas only 10-20% of the TAMs are monocyte-derived (TAM-MDM) (Fig. 2). Bioinformatic analyses revealed that metastatic colonization induces pronounced changes in transcriptomic programs in TAMs compared to their normal cellular counterparts. Transcriptomic programs of tumor education remain stable during tumor progression. Importantly, our data revealed a functional dichotomy with TAM-MG being associated with pro-

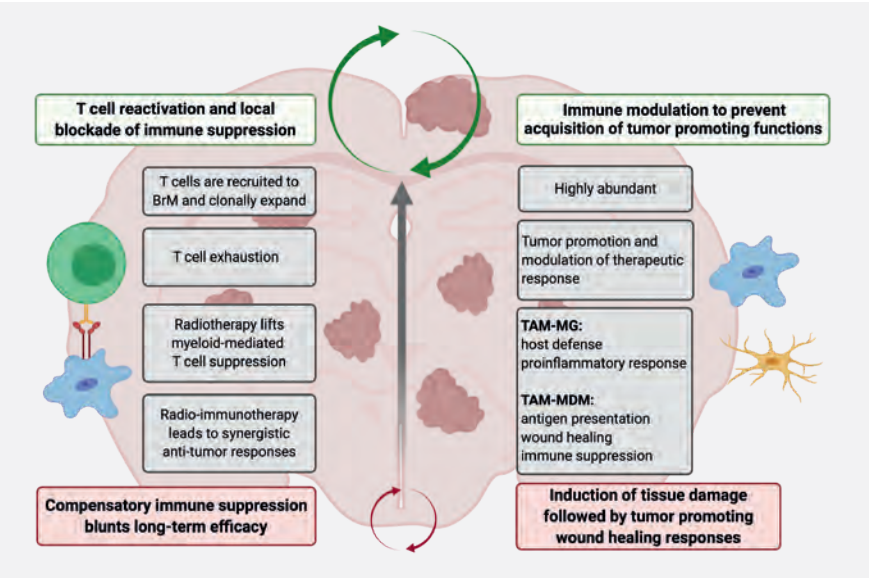
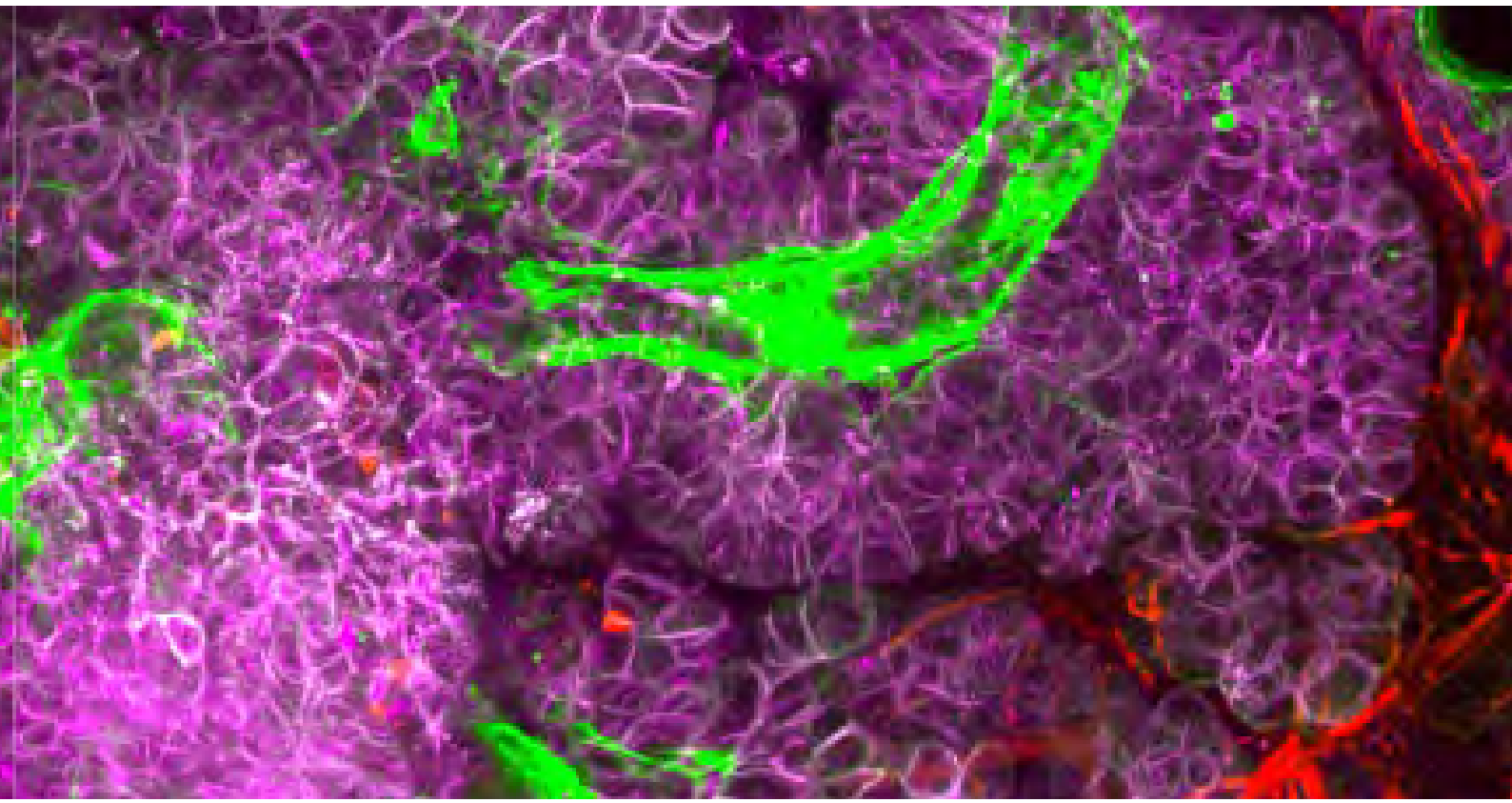


Figure 1.
Model figure summarizing key characteristics of the lymphoid and myeloid compartment in brain metastases. Lymphoid and myeloid-targeted therapies show limited efficacy in brain metastases due to tissue specific limitations to therapeutic efficacy. Combination therapies have to be developed 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.



inflammatory responses and host defense mechanism, whereas TAM-MDM are involved in immune suppression, wound healing responses and antigen presentation. While initial transcriptomic changes in response to metastatic formation are pronounced, we observed only minor effects of radiotherapy on TAMs in a celltype and dose-dependent manner (Figure 3). In this context, we found that radiotherpay in TAM-MG induces DNA damage responses, whereas TAM-MDM showed transient loss of tumor-education gene signatures.

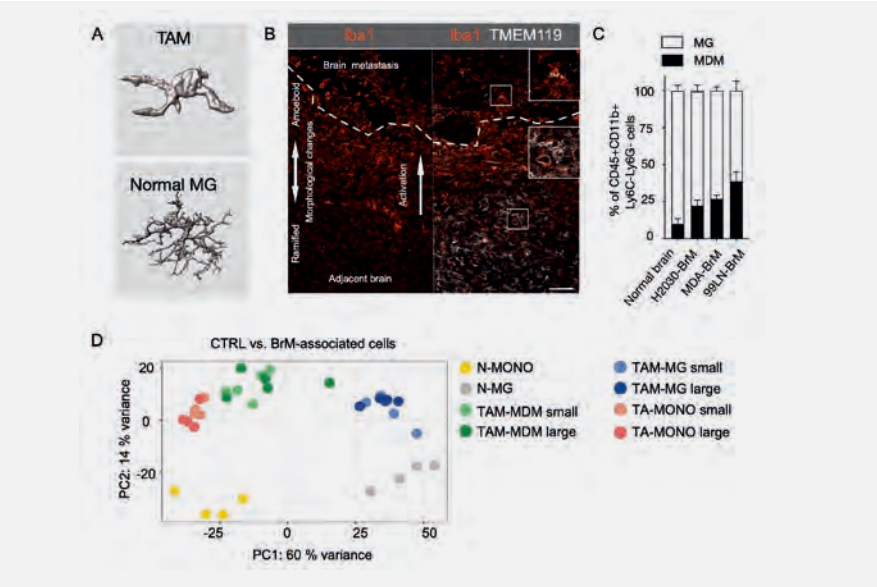
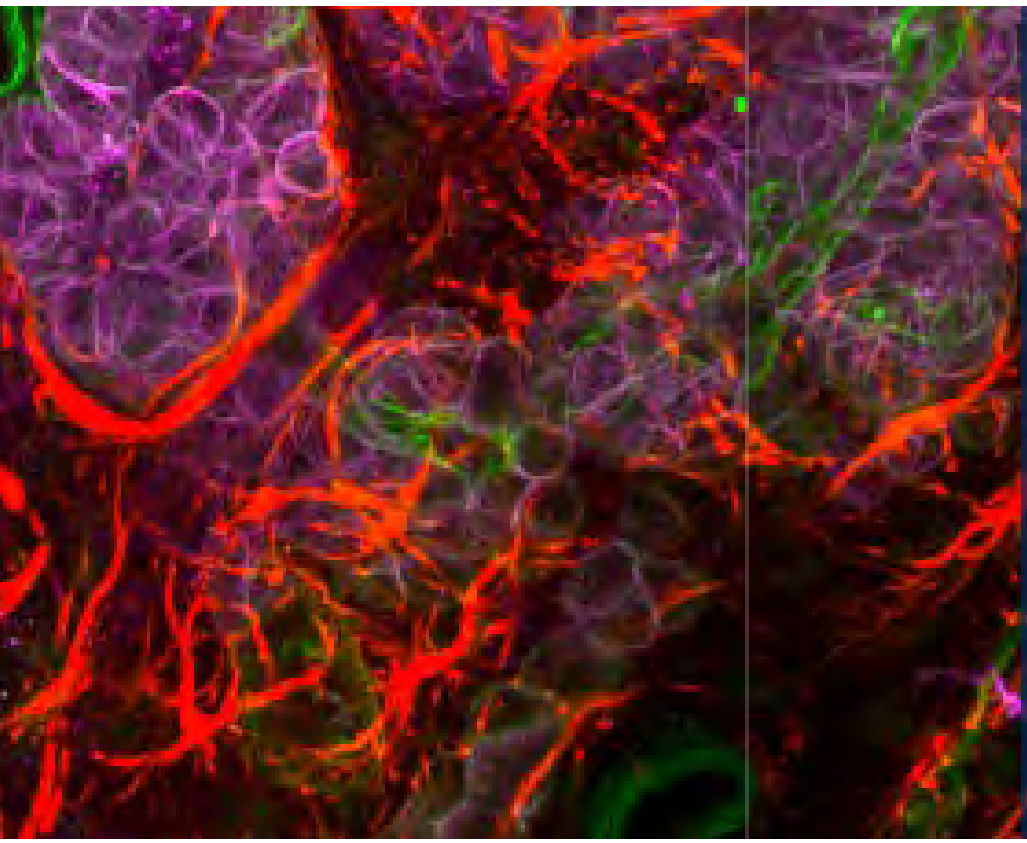


Figure 2.
(A) Tumor-associated macrophages (TAM) show morphological changes characteristic for highly activated microglia/macrophages compared to normal ramified microglia (MG). (B) Combination of Iba1 (macrophage/microglia marker) and TMEM119 (microglia marker) allows to distinguish TAM-MG and TAM-MDM in brain metastases. (C) Flow cytometric analyses confirms the presence of TAM-MDM in different brain metastases models. (D) PCA plot illustrates the transcriptional changes in tumor-associated myeloid compartment in a cell type specific manner with minor effects of different sizes of tumor lesions. Panel B and D are modified from Schulz et al., iScience 2020.

Radiotherapy as a sensitizer for tumor microenvironment-targeted and immunotherapies

The use of radiotherapy has largely been guided by the dogma that IR induces DNA damage leading to cell cycle arrest or cell death in rapidly proliferating cells. Apart from the notion that necrosis elicits inflammation due to the release of cellular content, radiotherapy has long been regarded as an immunologically inert process. While immunological effects of radiotherapy were neglected for decades, several discoveries, including abscopal effects and immunogenic cell death (ICD) established a close link between radiation and inflammation. Hence, radiation is increasingly regarded as a potent sensitizer of tumors towards immunotherapy (Figure 1). Indeed, there is accumulating evidence from clinical and pre-clinical studies, that the combination of radiotherapy and checkpoint inhibitors is more efficient than monotherapies. However, the underlying mechanisms of improved treatment



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efficacy of radio-immunotherapy remain unknown. We observed that radiotherapy can transiently lift the immune suppressive pressure in brain metastases most likely by enhancing the recruitment of tumor- and treatment naïve monocyte-derived macrophages to brain tumors that revert tumor-education gene signatures by replenishing the TAM pool. Moreover, our data indicate that radiotherapy also exerts effects on tumor infiltrating T cells which supports their reactivation in response to immune checkpoint blockade. Hence, effects of radiotherapy can modulate the myeloid and lymphoid compartment in brain metastases and help to overcome tissue-specific limitation to successful therapeutic intervention.

Novel concepts for tumor microenvironment-targeted and immunotherapies

Tumor associated macrophages/microglia are emerging as promising targets for tumor microenvironment-directed therapies. Major limitations of previously tested strategies stem from the inability to discriminate between brain-resident microglia and monocyte-derived macrophages. However, our data indicate that it is crucial to develop strategies that specifically disrupt cell type-dependent tumor promoting functions of TAMs. Strategies that prevent the induction of tumor-educated phenotypes by disrupting tumor cell-TAM interaction are therefore expected to be more efficient in controlling tumor progression compared to TAM depletion strategies that also affect important house keeping functions. We therefore seek to gain detailed insight into the communication between tumor cells and TAM-MG and TAM-MDM to unravel pathways that induce tumor education gene signatures in TAMs in BrM. This knowledge will be critical to develop therapeutic intervention strategies with the aim to (i) maintain host defense responses in TAM-MG or (ii) block tumor promoting, immune suppressive functions of TAM-MDM. In particular local and transient resolution of TAM mediated immune suppression could significantly improve the efficacy of T cell directed that are otherwise blunted by the immune suppressive pressure of the myeloid cell compartment.

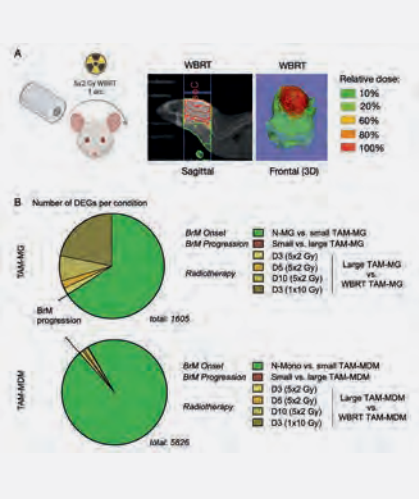

Figure 3.
Tumor progression and therapeutic intervention by whole brain radiotherapy (WBRT) shows only minor effects on transcriptomic programs in TAM-MG and TAM-MDM compared to the significant changes induced by initial tumor education. Panel B is modified from Schulz et al., iScience 2020.

III

Experimentelle Therapie
Experimental Therapy

CAR-exprimierende Lymphozyten für die adoptive Krebs-Immuntherapie

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Thorsten Geyer
Barbara Uherek

CAR-engineered lymphocytes for adoptive cancer immunotherapy

chimeric antigen receptors

natural killer cells

tumor microenvironment

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 together with one or more costimulatory protein domains. CAR-engineered T cells targeting CD19 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, but clinical experience with CAR-NK cells is still limited. NK cells are part of the innate immune system and play an important role in cancer immunosurveillance, with their cytotoxicity being triggered rapidly upon stimulation through germline-encoded cell surface receptors. In addition, NK cells modulate T-cell mediated antitumor immune responses by maintaining the quality of dendritic cells and enhancing the presentation of tumor antigens.

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 und Differenzierungsantigene wie CD19 und FLT3. 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).

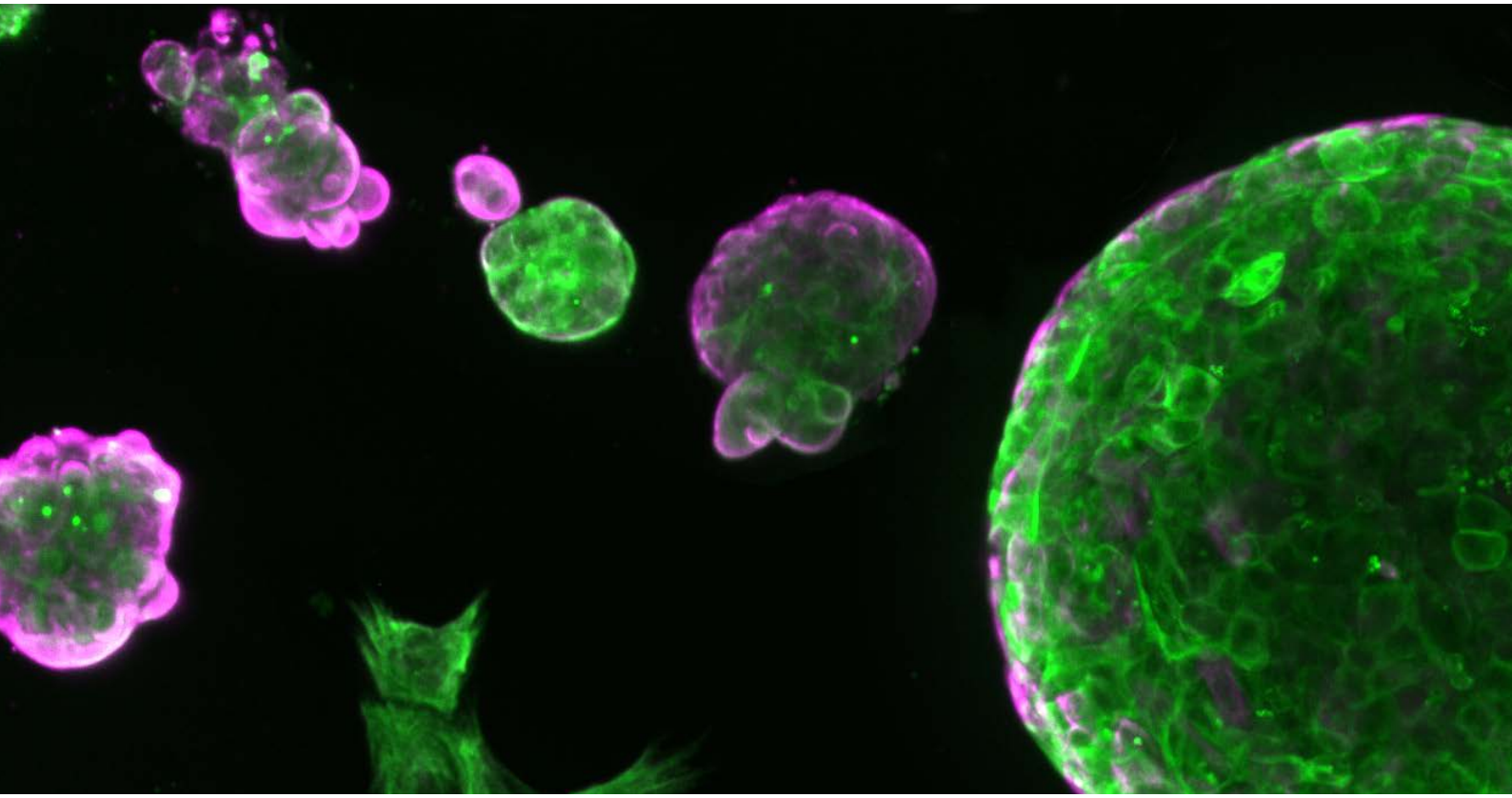
In cancer patients NK cells are often functionally compromised due to the immunosuppressive activity of the tumor. Hence, for adoptive cancer immunotherapy donor-derived allogeneic NK cells are preferred since they do not recognize tumor cells as 'self', thereby bypassing inhibitory signals.

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. To facilitate more flexible targeting of tumor cells, we generated in collaboration with the Institute of Radiopharmaceutical Cancer Research at the Helmholtz Zentrum Dresden-Rossendorf NK-92 cells that express a universal CAR (UniCAR) directed to a defined peptide epitope not naturally present on the surface of cells. For tumor-specific cell killing, these off-the-shelf UniCAR NK cells are combined with recombinant adapter proteins ('target modules') that contain a tumor-specific binding domain of choice, fused to the peptide epitope recognized by the UniCAR.

CAR-NK cells for clinical applications

In close collaboration with colleagues at the Institute for Neurooncology, the Department of Neurosurgery and the German Red Cross Blood Donation Service in Frankfurt, a protocol for a single center, open label 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 was designed (CAR2BRAIN; NCT03383978, clinicaltrials.gov). The dose escalation part of the CAR2BRAIN study has recently been completed (single dose injection into the wall of the resection cavity during relapse surgery). No dose-limiting toxicities were encountered at the applied dose levels, demonstrating safety and feasibility of our approach. Since summer 2020, patient recruitment for the expansion cohort is ongoing. These patients are scheduled to receive additional weekly injections of NK-92/5.28.z cells through an implanted catheter and reservoir. The clinical study is currently being expanded to a multicenter phase I trial including several other clinical centers within the German Cancer Consortium (DKTK)



network. As a prerequisite for extending this 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 (Figure 1).

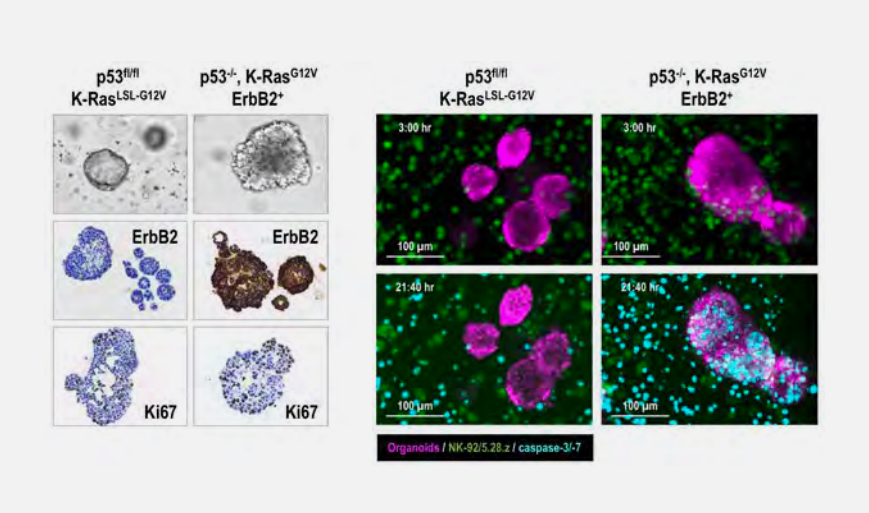
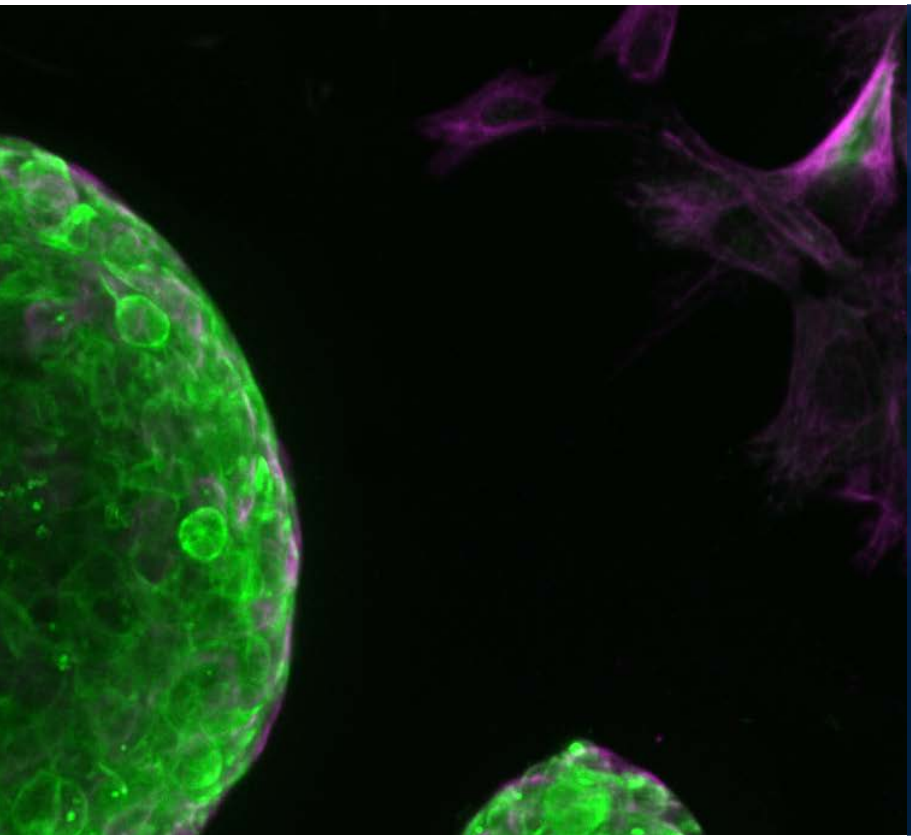


Figure 1.
Activity of NK-92/5.28.z CAR-NK cells against ErbB2-positive breast carcinoma organoids.
Mammary epithelial cells from Scgb1a1-Cre^{ERT2}/K-ras$^{LSL-G12VlacZ}$/Trp53$^{fl/fl}$ (SKP^{lacZ}) mice (kindly provided by Ernesto Bockamp, Universitätsmedizin, Mainz) were transduced with a lentiviral ErbB2-IRES-Cre vector to delete p53 and induce expression of K-Ras^{G12V} and ErbB2 (HER2). Morphological and molecular changes indicative of neoplastic transformation were assessed by bright field microscopy and immunohistochemistry of mammary gland organoids (left). Cell killing activity of ErbB2-specific NK-92/5.28.z CAR-NK cells (labeled with CFSE, green) against transformed ErbB2-positive mammary gland organoids (labeled with far-red Cell Tracer, pink) was analyzed by CQ1 confocal imaging of cultures kept in a collagen matrix over a 24 hours time period. Representative images from two time points are shown. Apoptosis induction in tumor cells was detected with a caspase-3/-7 probe (light blue). Also activated CAR-NK cells are transiently labeled due to granzyme B mediated conversion of the fluorescent caspase substrate.

CAR-engineered primary NK cells

Ex vivo differentiation of CAR-gene-transduced hematopoietic stem cells (HSCs) may be a feasible approach to generate off-the-shelf therapeutics based on primary CAR-NK cells. As a first step in this direction, we established a protocol that reproducibly allows to derive mature and functional CD56$^+$ NK cells from mobilized peripheral blood HSCs. To support more pronounced cell expansion, we generated K562 feeder cells co-expressing CD137 ligand (4-1BB-L) and membrane-anchored IL-15 and IL-21. Co-culture of peripheral blood-derived as well as *ex vivo* differentiated NK cells with these feeder cells dramatically improved NK cell expansion and compensated for donor-dependent variability seen during cytokine-based expansion (Figure 2). Currently, this approach is being tested for genetically engineered HSCs.



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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 glioblastoma models in immunocompetent mice, 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 immunocytokines which harbor a PD-L1-specific antibody domain, fused to an IL-15 superagonist or single-chain IL-12. Secretion of such molecules by CAR-NK cells and retention within the tumor microenvironment by binding to PD-L1 on cancer cells can simultaneously block the PD-1/PD-L1 immune checkpoint and provide high local concentrations of the cytokines to support the antitumor activity of CAR effector cells and bystander immune cells.

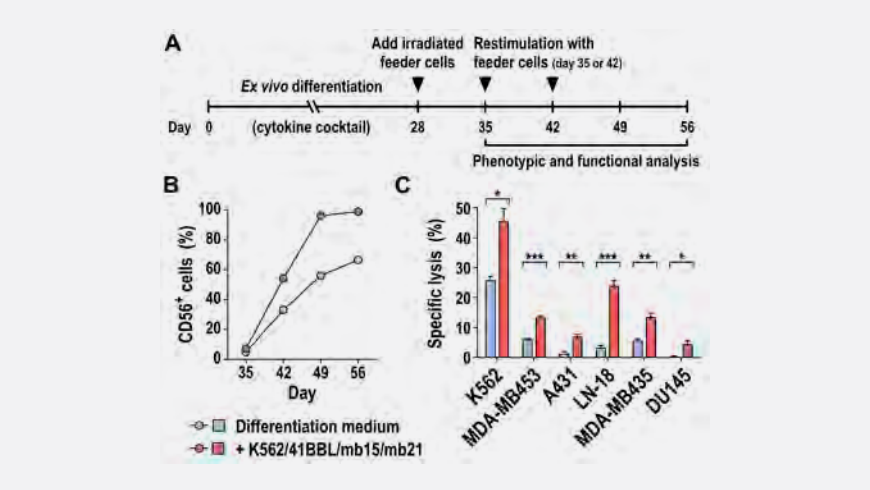

Figure 2.
Two-step process for NK cell generation from hematopoietic stem and progenitor cells. (A) Mobilized CD34+ HSCs were first cultured for four weeks in cytokine-containing medium inducing NK-cell differentiation. On day 28, cells from the differentiating cell pool were mixed with irradiated K562/41BBL/mb15/mb21 feeder cells. (B) Starting after one week of co-culture (day 35), the development of CD56+ NK cells was analyzed once weekly by flow cytometry. For comparison, CD34+ cells cultured in differentiation medium without feeder cells were included. (C) Antitumor activity of NK cells differentiated in cytokine-containing medium with or without addition of feeder cells as shown in (A) was tested with K562 erythroleukemia, MDA-MB453 breast carcinoma, A431 squamous cell carcinoma, LN-18 glioblastoma, MDA-MB435 melanoma and DU145 prostate cancer cells as targets in co-culture experiments at an effector to target ratio of 5:1. Mean values ± SEM of triplicate samples are shown. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. In (B) and (C) cells from the same representative donor were used.

Zentrale Einheit Transgenic Core

Transgenic Core Facility

Gene Editing Labor und Zentrale Einheit Transgenic Core

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Genetically engineered mouse models (GEMMs)

CRISPR and Cre/loxP

genome editing

mouse model generation

Recent advances in genome editing, in particular the CRISPR/Cas9 technology, have revolutionized the generation of mouse models. Combination of CRISPR, homology-directed targeting, and Cre/loxP open novel opportunities in modelling biological processes. Generating GEMMs with relevant in vivo readouts requires both: expertise in genetic strategies and construct assembly, and access to microinjection expertise.

Gene Editing and Transgenics integrate fundamental steps in the GEMM generation process, such as genetic design of the desired in vivo model, construct development and validation, and delivery methods to ensure high-impact mouse models. Gene Editing Laboratory applies its expertise and comprehensive experience in allele design and targeting, CRISPR/Cas9 and Cre/loxP, genome editing of cells and embryos, while TCF specializes on embryo delivery methods. As both fertilized oocytes and embryonic stem cells can be modified genetically, we exploit targeted CRISPR techniques to advance the GEMM generation.

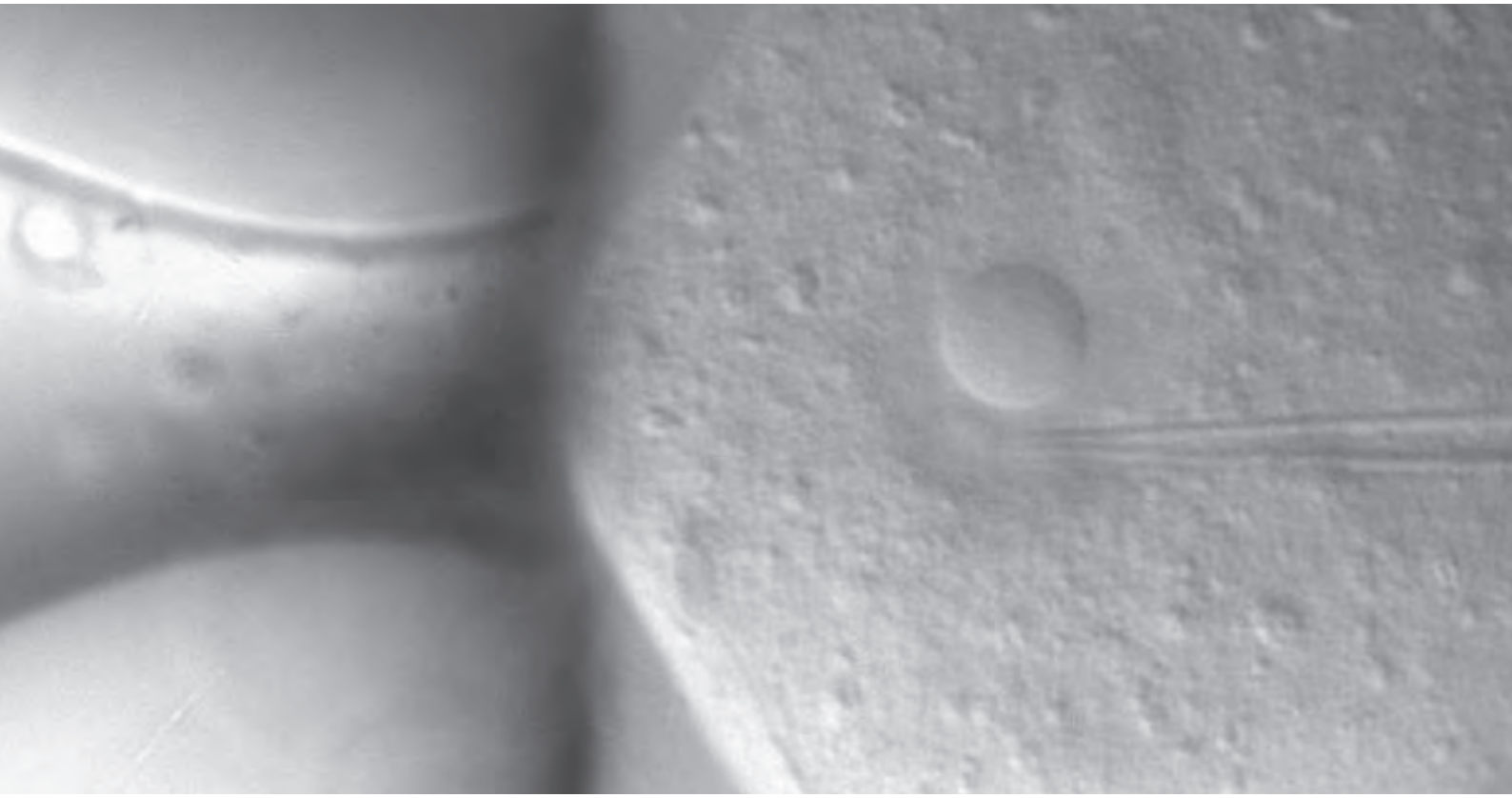
Das Gene Editing Labor (GEL) und die Transgenic Core Facility (TCF) am Georg-Speyer-Haus generieren erfolgreich neue Mausmodelle. Dr. Madina Karimova entwickelt Strategien für gentechnisch veränderte Mausmodelle (GEMMs) und setzt die Generierung der Mausmodelle mit ihrem Team um. Gene Editing Labor erstellt ab *initio* neuartige CRISPR-Modelle, wie z. B. Gen-Knockout, Punktmutationen, Patienten-Allele, präzise Deletionen, Protein-Verkürzungen und andere. Um eine robuste CRISPR-Effizienz in den Eizellen sicherzustellen, hat TCF hochwertige Embryo-Reagenzien und -Protokolle entwickelt (Cas9 RNP, stabilisierte sgRNA- und HDR-Spender, lange ssDNA) und hocheffiziente Abgabemethoden wie die Batch-Embryo-Elektroporation. Routinemäßig F1-Sequenz-validierte CRISPR-Modelle werden innerhalb weniger Monate abgeschlossen. Mehrere CRISPR-Modelle und Chimären wurden in 2020 erfolgreich generiert.

Das Gene Editing Laboratory entwickelt genetische Strategien für das gewünschte in-vivo Modell und führt innovative Forschungen zu Gen-Editing-Technologien durch. Im Jahr 2020 wurden für fortschrittliche Mausmodelle ein neuartiges far-red fluoreszierendes Proteinreporter Konzept und eine genetische Selbstentfernungs-Strategie mit Vika/Vox Rekombinase entwickelt. Für Kollaborations-Partner entwickelt GEL Strategien für komplexe genetische Mausmodelle wie Cre-induzierbare gewebespezifische Punktmutationen, Multiplexed Lineage Tracing, neuartige Cre-Treiber und induziertes in-vivo-Gen-Silencing in Verbindung mit In-vivo-Zell-Tracing.
TCF hat als Serviceeinheit das Kryokonservierungsprogramm weiter ausgebaut und GSH-Gruppen durch Spermien-Kryokonservierung unterstützt. Die IVF-basierte Linien-Rederivation war in TCF für C57Bl6J- und NSG-Hintergrundlinien ebenfalls erfolgreich.

Our primary focus lies on the latest know-how in CRISPR genome editing and in devising the best strategies. We create novel models ab *initio*, such as gene knockouts, point mutations, patient alleles, precise small and large deletions (>10kb), and protein truncations. To ensure robust CRISPR efficiencies in the oocytes, we have developed high-quality embryo reagents and protocols (Cas9 RNP, stabilized sgRNA and HDR donors, long ssDNA), and high-efficiency delivery methods, such as batch embryo electroporation. Our CRISPR models provide 100% germline transmission and are analyzed for mosaicism. Routinely, F1 sequence validated CRISPR mice are generated within few months. Multiple CRISPR mouse models and chimera-based GEMMs were generated in collaboration with internal and external research groups in 2020.

Gene Editing Laboratory develops genetic strategies for the desired in vivo model and carries out innovative research in gene editing technologies. In 2020 a novel far-red fluorescent protein reporter and self-excision Vika/vox strategies have been developed for advanced mouse models. For our collaborations, GEL devises strategies for complex genetic mouse models, such as Cre-inducible tissue-specific point mutations, multiplexed lineage tracing, novel Cre-drivers, and induced *in-vivo* gene silencing coupled to *in vivo* cell tracing.

TCF as a service unit has further expanded the cryopreservation program and supported GSH groups with sperm cryopreservation (> additional 30 strains). IVF-based line rederivation was successful in TCF for C57Bl6J and NSG background lines to support GSH groups *in vivo* research.



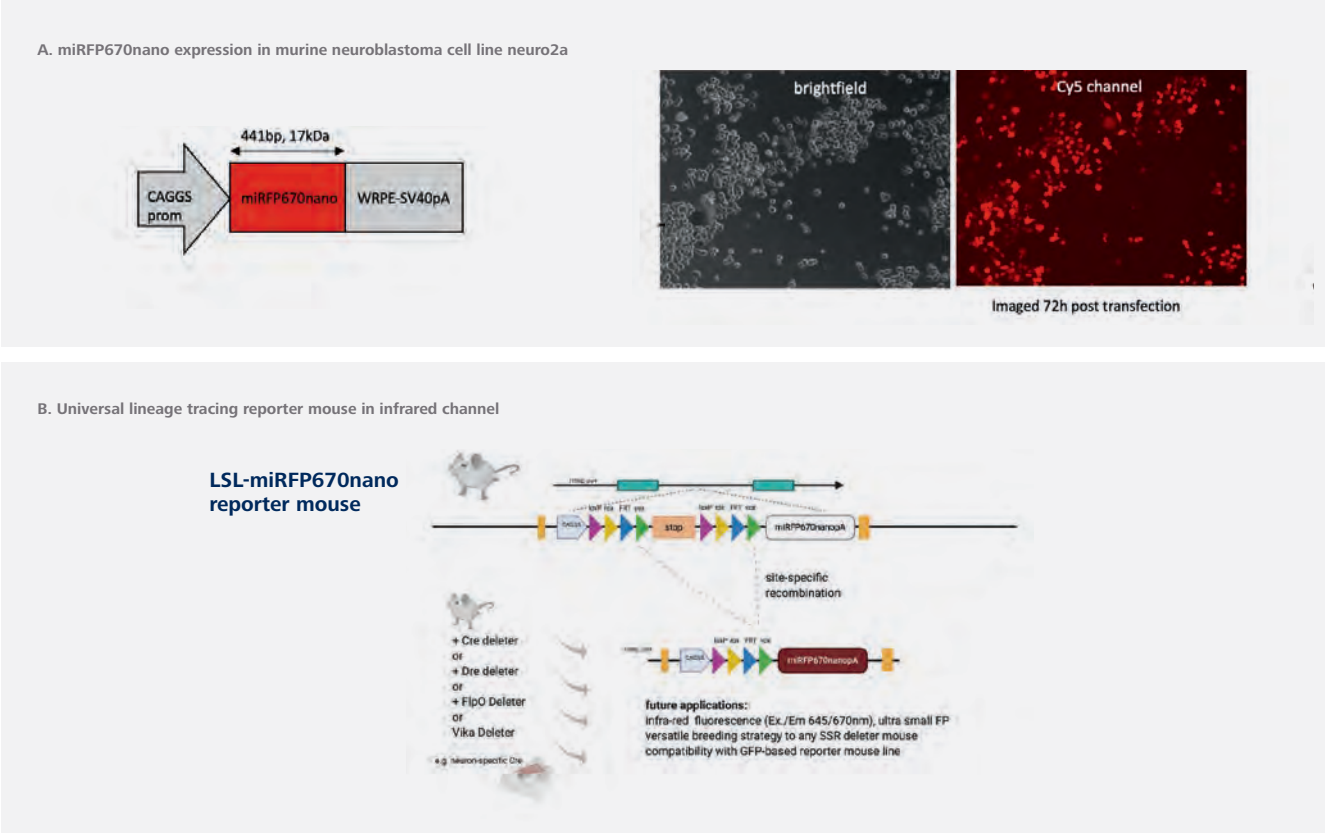
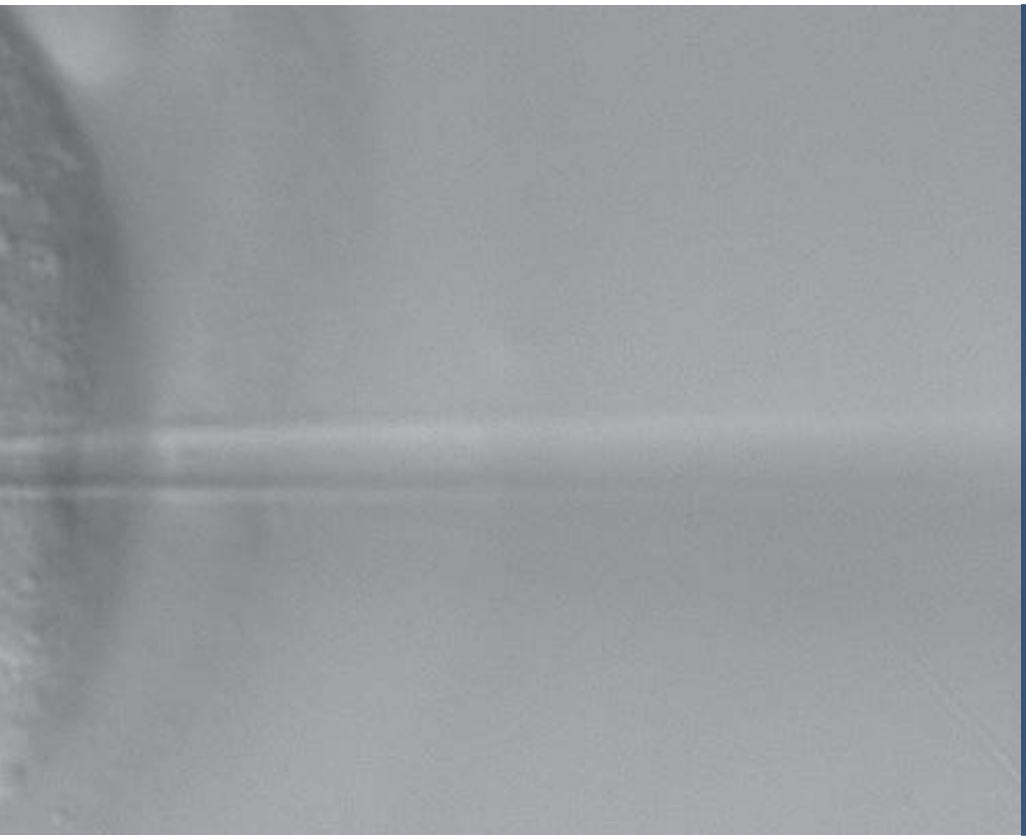


Figure 1.
Novel reporter mouse model development with conditionally inducible fluorescence in near-infra-red spectrum. A. Cloning and expression in mammalian cells of the novel fluorescent protein developed from cyanobacteriochrome photoreceptor (Oliinyk et.al. Nat Comm, 2019). B. Schematic representation of the novel mouse model with multiplexed conditional lox-stop-lox cassette for deep tissue imaging. Currently in development by Gene Editing Laboratory.



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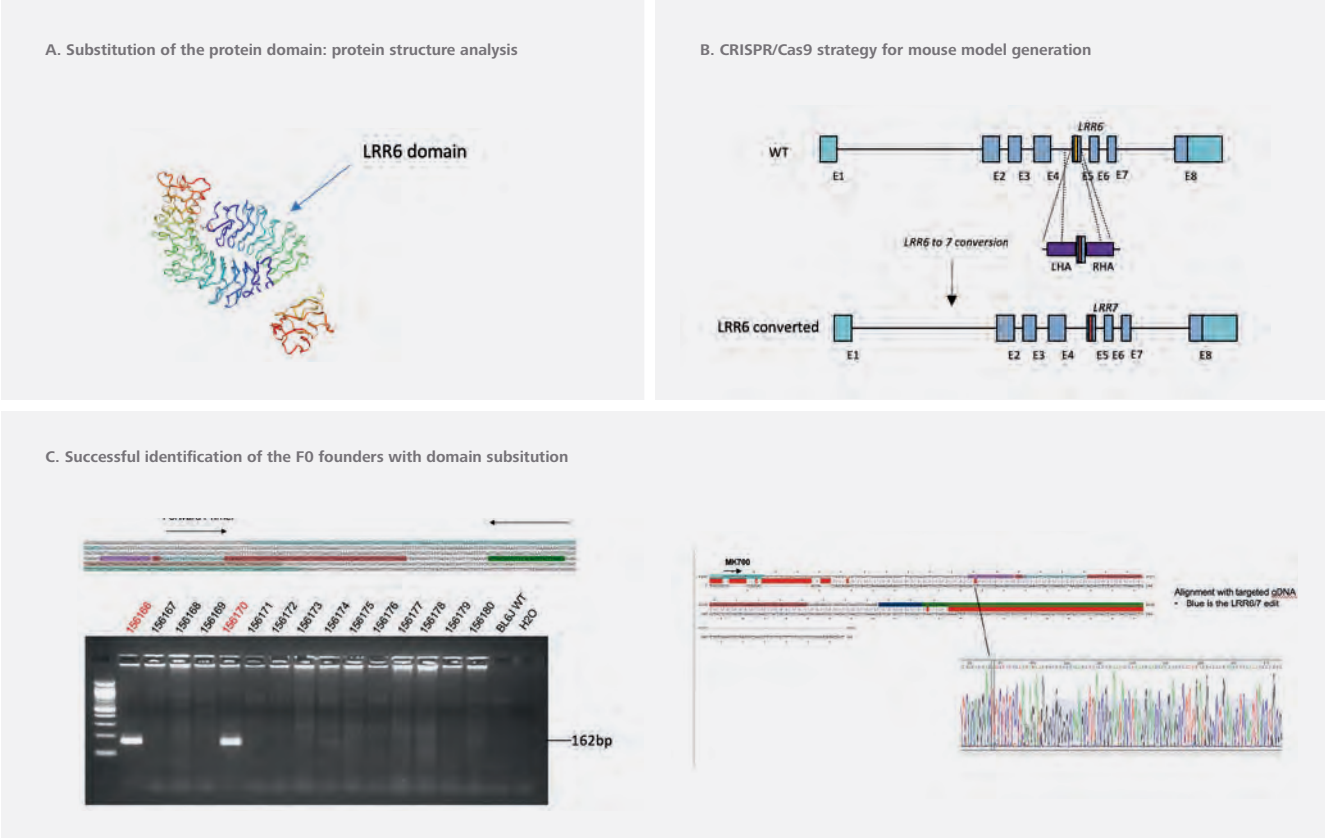

Figure 2.
CRISPR/Cas9 assisted mouse model generation. A. Identification and analysis of the domain of interest on protein level is crucial for genetic planning of the in-vivo mouse model and human-to-mouse sequence translation. B. Genetic strategy of the CRISPR-based domain conversion. Wildtype sequence is cleaved in oocytes by Cas9 and gRNA specific to the locus and HDR-based sequence substitution is achieved by ssODN supplementation. C. Identification of the F0 founder animals by PCR genotyping with primers specific to novel converted allele. Bottom panel: DNA alignment and DNA chromatogram of the Sanger sequencing, confirming correctly introduced sequence in the founder animal.

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Reviews and Editorials

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Akademische Ausbildung

Julian Anthes
"Effects of macrophage depletion on radio-immunotherapy in breast-to-brain metastasis". Masterarbeit im Studiengang Molekulare Medizin, Goethe Universität Frankfurt 2020

Jessica Kondol
„Investigation of the role of tumor-associated macrophages in the extravasation of brain metastatic tumor cells". Masterarbeit, Technische Universität Darmstadt 2020

Aylin Möckl
"Exploring the role of CEACAM1 and IGFBP4 for brain tropism in breast-to-brain metastasis". Masterarbeit im Studiengang Molekulare Medizin, Goethe Universität Frankfurt 2020

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Akademische Ausbildung

Sophie-Christin Linkenbach
„Generierung von Feeder-Zelllinien für die selektive Expansion muriner NK-Zellen", Masterarbeit im Fachbereich Medizin, Studiengang Molekulare Medizin der Goethe-Universität Frankfurt, 2020.

Aline Häcker
„Immunzytokine zur Steigerung der antitumoralen Aktivität von CAR-modifizierten Lymphozyten", Doktorarbeit im Fachbereich Biologie der Technischen Universität Darmstadt, 2020.

Finanzen und Administration

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Die Abteilung Finanzen/Administration setzt jährlich ein Finanzvolumen von etwa 10 Millionen Euro um und betreut dabei rund 100 Mitarbeiterinnen und Mitarbeiter. Sie wird seit September 2019 von Franziska Hasslinger-Pajtler geleitet, die dabei von Robert Dornberger unterstützt wird. Besonderer Fokus der Administration liegt neben den klassischen Kernaufgaben einer jeden Verwaltung aktuell auf den Themen Gleichstellung, Korruptionsprävention, Compliance, Vereinbarkeit von Beruf und Familie, Digitalisierung und Datenschutz. Neu im Team ist Belinda Gehrmann, die die kaufmännische Leitung in allen Belangen unterstützt.

Christiane Bormann-Strack im Personalbüro bearbeitet alle Themen der Personalsachbearbeitung. Ilka Graus Fokus in der Finanzbuchhaltung/Drittmittelverwaltung liegt auf der Betreuung der Projektfördermittel des Bundes. Gabriele Heckl erstellt die Bilanz, bearbeitet alle Ausgangsrechnungen, die Reisekostenabrechnungen und ist für die Abrechnung internationaler Drittmittel zuständig. Giuseppina Virgillito betreut die Kreditorenrechnungen sowie die Drittmittel nationaler Stiftungen.

Ansprechpartner in der Telefonzentrale und am Empfang ist Bernd Würdemann. Adrian Gresik ist verantwortlich für die vielfältigen Aufgaben des Innendienstes. Er, Michael Paul und Heinrich Krompiec kümmern sich um die haustechnischen Ausstattungen und Installationen und unterstützen zudem bei der Organisation von wissenschaftlichen Tagungen und Veranstaltungen.

Yoseph Alazar, Yasemin Piskin und Neriman Sarac reinigen die Laboratorien, entsorgen die anfallenden Abfälle und kümmern sich um die Bereitstellung von Laborbedarf. Sie werden dabei von Keziban Ata unterstützt.

Since September 2019 our Administration and Services Department is managed by Franziska Hasslinger-Pajtler who oversees a yearly budget of approximately 10 million Euros. She is supervising the administrative needs of about 100 staff members and is supported by Robert Dornberger. In addition to the conventional administrative tasks, they are currently focusing in particular on Digitization, Data Protection, Equality, Prevention of Corruption, Reconciliation of Family and Working Life and Compliance. Team newcomer is Belinda Gehrmann who is assisting the commercial management in all matters.

Christiane Bormann-Strack heads the personnel department. Ilka Grau is engaged in financial accounting and the administration of Federal external research fundings. Gabriele Heckl prepares the balance sheets, is responsible for all outgoing invoices, all claims for travel expenses and is in charge of accounting of international grants. Giuseppina Virgillito handles funds grants from nationwide foundations and takes care of all incoming invoices.

Bernd Würdemann is our receptionist and is the first contact with the Institute. Adrian Gresik is responsible for various internal service tasks. Together with Michael Paul and Heinrich Krompiec, he takes care of the technical equipment, various constructions and installations, as well as makes preparations for all kinds of scientific meetings and events.

Yoseph Alazar, Yasemin Piskin and Neriman Sarac clean the laboratories, dispose generated waste and restock the laboratory supplies. They are supported by Keziban Ata.

Wissenschaftlicher Service

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Tierhaltung

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 Mitarbeiterinnen 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 nach 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 Biolumineszenz- 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.

Animal Husbandry

The 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 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).

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Zentrale Einheit Histologie
Zur Anfertigung von histologischen Präparaten betreibt das Georg-Speyer-Haus eine Histologie-Serviceeinheit unter der Leitung von Dr. Birgit Ritter. Hier werden von Petra Dinse und Natalia Delis, 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. Das Labor stellt seine Leistungen den Arbeitsgruppen des Georg-Speyer-Hauses sowie externen Forschergruppen zur Verfügung.

Core Facility Histology
The Georg-Speyer-Haus operates a histology core facility. It is supervised by Dr. Birgit Ritter. Petra Dinse and Natalia Delis are 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. The services of the histology core facility are available to all scientists of the Georg-Speyer-Haus as well as to external research partners in collaboration.

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Zentrale Einheit Durchflusszytometrie
Die zentrale Zytometrie-Einrichtung besteht aus drei Durchflusszytometern zur Zellanalyse (BD LSRFortessa, FACSCantoII, FACSCalibur) und zwei Zellsortern (BD FACSAriaI, FACSAria Fusion). 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. Annette Trzmiel führt die anfallenden Hochgeschwindigkeits-Zellsortierungen durch und ist für den einwandfreien Zustand aller Durchflusszytometrie-Geräte am Institut verantwortlich. In einigen Fällen fungiert Thorsten Geyer als zusätzlicher Operator an den Zellsortern. Die Sortiereinheit steht primär den Arbeitsgruppen des Georg-Speyer-Hauses, aber auch externen Forschergruppen zur Verfügung. Zusätzlich werden in der Einrichtung ein CQ1 Confocal Quantitative Image Cytometer (unter der Aufsicht von Dr. Tijna Alekseeva) zur 3D High-Throughput Zellanalyse sowie ein Bioplex200 zur Multiplex-Analyse betrieben.

Flow Core Unit (FCU)
The Flow Core Unit (FCU) of the Georg Speyer Haus operates three flow cytometer instruments (BD LSRFortessa, FACSCantoII, FACSCalibur) and two cell sorters (BD FACSAriaI and BD FACSAria Fusion). 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. Annette Trzmiel is responsible for high-speed cell sorting as operator in this central service unit for all research groups of the GSH as well as for external researchers. 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. In addition, a CQ1 Confocal Quantitative Image Cytometer (supervised by Dr. Tijna Alekseeva) for 3D high-throughput cell measurements as well as a Bioplex200 for multiplex analyses are installed in the facility.

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Geräte und Biologische Sicherheit
Dr. Stefan Stein berät bei der Beschaffung der nötigen Laborausstattung / 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 equipment and supply. As biosafety officer, he is responsible for biological safety at the institute and for communication with respective authorities.

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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-Projektmanagements, der Netzwerkadministration und des Einkaufs wahr. Er wird dabei unterstützt von Claudia Stein.

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. He is supported by Claudia Stein.

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Gefahrstoff- und Abfallmanagement
Dr. Klaus Lehmen hat die Verantwortung für den Bereich Gefahrstoff- und Abfallmanagement sowie den Aufbau eines digitalen Betriebsmittelkatasters.

Hazardous substances and waste manegement
Dr. Klaus Lehmen is in charge with managing of hazardous goods and disposals and implementing a digital register of all technical equipment and documents.

Hana Kunkel
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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.

Facility- / Lab Management
Hana Kunkel ensures the compliance with the current laboratory standards and safety regulations. She is responsible for managing all Cleaning services.

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
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100,– €

Studenten
Students
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150,– €

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Sponsor
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www.georg-speyer-haus.de/friends/index.htm

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„Gesundheit ist gewiss nicht alles,
aber ohne Gesundheit ist alles nichts.“
Arthur Schopenhauer