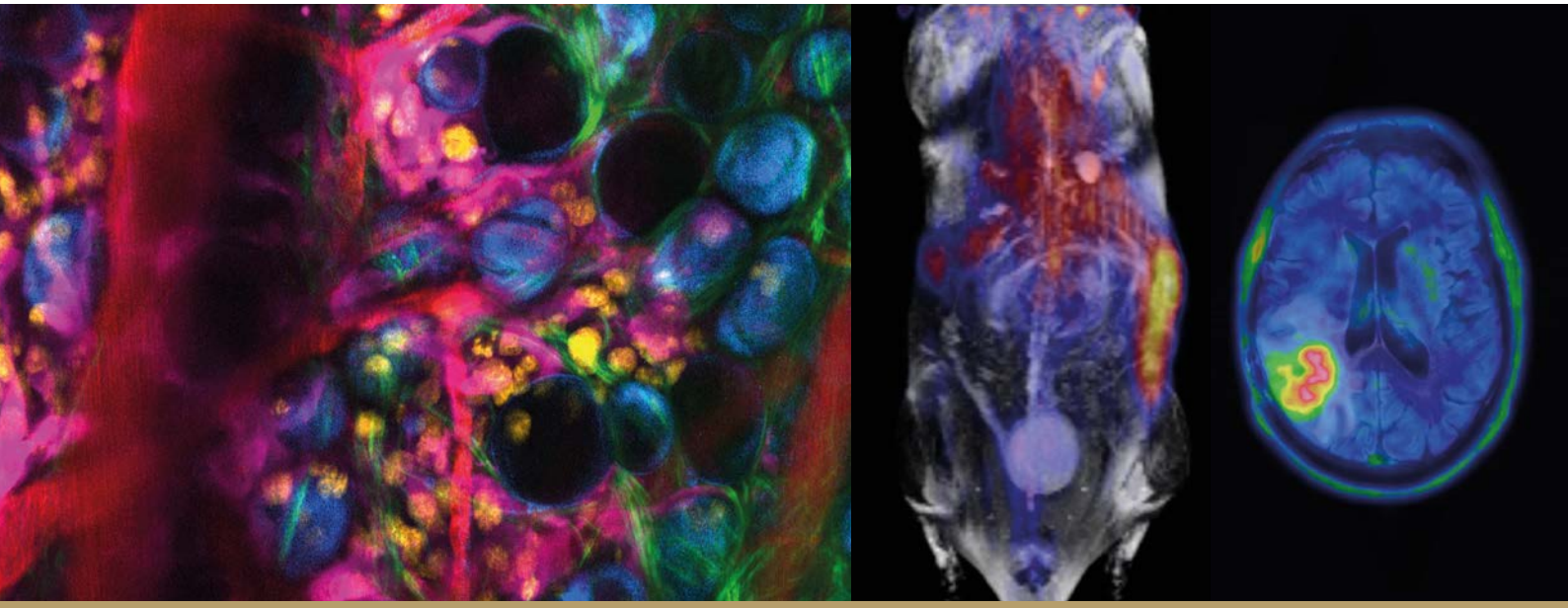
EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN



Department of

PRECLINICAL IMAGING & RADIOPHARMACY

WSIC
Werner Siemens
Imaging Center

Department of

PRECLINICAL IMAGING & RADIOPHARMACY

CONTENTS

WSIC AT A GLANCE

NATIONALITIES:
19
(as of Nov. 2021)

PATENTS:
10
(filed & accepted)

FUNDS:
>78 Mio. €
(since 2005)

GENDER RATIO:
50 % : 50 %
(as of Nov. 2021)

EMPLOYEES:
90
(as of Nov. 2021)

TRACER:
23
(for diagnosis & therapy)

RESEARCH GROUPS:
14

DOCTORAL THESES:
33
(successfully completed by Nov. 2021)

PUBLICATIONS:
> 356
(peer-reviewed publications)

01

OUR MISSION

IMAGING SCIENCE: ADVANCING BIOMEDICAL RESEARCH, ACCELERATING CLINICAL TRANSLATION AND ENABLING THERAPY GUIDANCE

Imaging science is an emerging field that impacts various biomedical research areas, such as oncology, immunology, neurology and infectious diseases.

Noninvasive imaging methods, such as magnetic resonance imaging (MRI) and positron emission tomography (PET), allow the direct *in vivo* quantification of functional processes, molecular markers and metabolic rates using target- or disease-specific contrasts and radiolabeled tracers. Thus, imaging can replace time-consuming and less reliable *ex vivo* and *in vitro* methods in many areas of biomedical science and clinical diagnosis.

In addition to the contributions of noninvasive imaging to academic research and clinical diagnosis, the pharmaceutical industry also benefits from these tools. Imaging can accelerate drug and biomarker development by yielding more reliable *in vivo* results, enabling cost-effective study designs, and reducing the numbers of animals required for experiments. Consequently, the pharmaceutical industry can more rapidly advance products to the market and positively impact animal protection.

Preclinical imaging allows easy translation of results from the laboratory bench to the clinic. The latest advances in theranostics and image-guided tumor therapies provide new directions for innovative clinical research and enable new therapeutic strategies.

An interdisciplinary team of highly motivated and skilled biologists, physicists, physicians, chemists, biochemists, engineers, technical assistants and lab managers form the Werner Siemens Imaging Center and the Department of Preclinical Imaging and Radiopharmacy, one of five Departments in Radiology at the University Hospital Tübingen (UKT), and pioneer preclinical and translational imaging science. The Werner Siemens Imaging Center has evolved from a small laboratory into a leading international imaging science center that performs cutting-edge research in the fields of oncology, immunology, neurology and infectious diseases. The interdisciplinary nature of our team is reflected in the variety of research areas studied by the Werner Siemens Imaging Center. We invite you to read the chapters of this brochure to learn about our research and the fascinating options that preclinical and molecular imaging offers.

Professor Dr. Bernd Pichler
Chair & Director
Department of Preclinical Imaging and Radiopharmacy
Werner Siemens Imaging Center

IMAGING SCIENCE: AN EMERGING TOOL FOR TRANSLATIONAL RESEARCH AND PRECISION MEDICINE

The mission of the Werner Siemens Imaging Center is to bridge the gap between *in vitro* biomedical research and *in vivo* imaging. This goal is achieved by developing novel imaging technologies and innovative imaging probes.

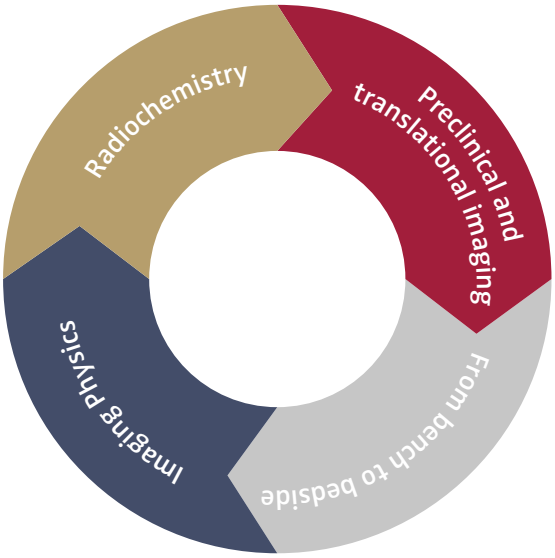
The lab utilizes the latest technological infrastructure and sets the highest standards in animal hygiene, animal welfare and physiological monitoring of animals. The large number of established imaging protocols, standard operating procedures (SOPs), and data analysis tools enable innovative research and guarantee reliable scientific results.

A close connection to the University Hospital Tübingen enables translational research and early clinical studies and ensures rapid transition of expertise from the research laboratory to patient bedsides. Technologies, such as PET/MR or novel PET tracers, developed at the Werner Siemens Imaging Center have been successfully translated to the clinics.

Universitätsklinikum Tübingen

Our radiopharmacy group produces patient-individualized diagnostic markers for PET imaging under good manufacturing practice (GMP) conditions to yield reliable and innovative diagnostic options for our patients.

Most human diseases are characterized by a complex interplay of multiple physiological and pathophysiological factors. Such multicausal events require precise diagnoses and patient-individualized therapies. Noninvasive imaging using specific markers delivers holistic information about disease spread, disease phenotype and disease progression, thus forming an important cornerstone for current and future health care strategies. With this objective, maintaining a comprehensive imaging platform with novel disease-specific imaging markers, advanced imaging technologies and standardized imaging data analysis tools is essential. This complex interplay requires an interdisciplinary team of preclinical and clinical scientists who form a strong alliance to develop future health care strategies for precision and personalized medicine.



02

WERNER SIEMENS IMAGING CENTER

PRECLINICAL IMAGING AT TÜBINGEN

... funded by the Werner Siemens Foundation

The Werner Siemens Foundation was established in Schaffhausen in 1923 to preserve the values of social responsibility and integrity held by the Werner brothers, Carl and William. The spirit of mutual responsibility was deeply instilled in the family and shared by the brothers, as owners of the family business, with the ever-increasing number of employees.

In 1955, the board of trustees announced the official endorsement of the Foundation. The Foundation, in which the family is directly represented through its board, has flourished since that time.

The activities of the Foundation are subdivided into community services and family foundation tasks for emergency aid. Currently, the Foundation oversees 400 of the 500 descendants of the 6th generation of Carl and Werner von Siemens. The Foundation promotes projects in both public and private institutions in the fields of education, science, health care, nature, culture and youth support. The Foundation's contribution to Siemens' share capital is approximately three percent.

The Laboratory for Preclinical Imaging and Imaging Technology and the associated endowed chair at the Eberhard Karls University Tübingen have been funded by the Werner Siemens Foundation since 2007. At that time, the laboratory was relatively small, with only 12 members. To mark its 90th anniversary in 2012, the Foundation donated an additional eight million euros, enabling the expansion of the existing laboratory infrastructure and the acquisition of state-of-the-art imaging technology. As a result of the extensive and sustainable funding provided by the Foundation, the laboratory has since grown to over 90 members. In recognition of the strong and lasting relationship among the laboratory, the University, and the Foundation, the Rector and the Medical Faculty of the University of Tübingen decided to establish the Werner Siemens Imaging Center in 2014. In October 2016, the University of Tübingen recognized the Foundation's commitment to and extensive support of preclinical imaging and awarded the 2016 University Prize to the Werner Siemens Foundation.

The new research building and Werner Siemens Imaging Center, funded by the Werner Siemens Foundation, was inaugurated and officially opened on November 21st, 2014. It offers 637 m^2 of imaging research labs, 626 m^2 of state-of-the-art organic chemistry and radiochemistry research labs, 233 m^2 of GMP labs for radiopharmaceutical production and 472 m^2 of office space.

Werner von Siemens
1816 - 1892

WSS
WERNER SIEMENS-STIFTUNG

iFIT – CLUSTER OF EXCELLENCE

iFIT – Image-Guided and Functionally Instructed Tumor Therapies

Prof. Bernd Pichler and the Department of Preclinical Imaging and Radiopharmacy are highly involved in the Image-Guided and Functionally Instructed Tumor Therapies (iFIT) Cluster of Excellence granted to the Eberhard Karls University Tübingen by the German Research Foundation (Deutsche Forschungsgemeinschaft – DFG). The iFIT Cluster of Excellence brings together world-leading expertise in three important areas of cancer research that to date have been largely separate. When these areas of expertise are brought together, they promise an unprecedented level of understanding of tumor biology and facilitate the development of novel diagnostic and therapeutic approaches for the treatment of cancer.

THESE AREAS INCLUDE:

A. the identification of 'functionally instructed molecular therapies' via functional genomics,
B. the combination of these therapies with and further development of advanced immunotherapies and
C. the use of multiparametric imaging techniques to guide tumor therapies and to reveal novel classes of therapeutic targets.

iFIT scientists will particularly focus on biological processes that enable tumors to survive under cellular stress. State-of-the-art imaging techniques will be used to visualize the stress states and stress responses of tumors, enabling the application of novel molecular targeted, novel immuno- or combinatorial therapies in an image-guided and personalized context. The spokesmen of the cluster are the oncologist Prof. Lars Zender, Medical Director of the Department of Internal Medicine VIII – Medical Oncology & Pneumology; Prof. Bernd Pichler, Director of the Werner Siemens Imaging Center; and Prof. Hans-Georg Rammensee, Chair of the Department of Immunology.

Additional institutions participating in the cluster are the Max Planck Institutes for Developmental Biology and Intelligent Systems, the Natural and Medical Sciences Institute at the University of Tübingen, and the Margarete Fischer Bosch Institute for Clinical Pharmacology.

In addition to his position as a spokesman, Prof. Bernd Pichler is a coordinator of research area C together with Prof. Christian la Fougère, Prof. Konstantin Nikolaou and Prof. Bernhard Schölkopf. Collaborative iFIT research projects are performed together with our research groups in Oncology, Advanced Preclinical Metabolic Imaging and Cell Engineering, Preclinical Imaging of the Immune System, and Imaging Probe Development. **The iFIT-associated PIs of the Werner Siemens Imaging Center include Dr. Manfred Kneilling, Dr. Julia Mannheim, Prof. Dr. André F. Martins, Dr. Andreas Maurer, Apl. Prof. Dr. Gerald Reischl, Dr. Andreas Schmid, Dr. Johannes Schwenck, MD, PhD and Prof. Dr. Bettina Weigelin.**

iFIT

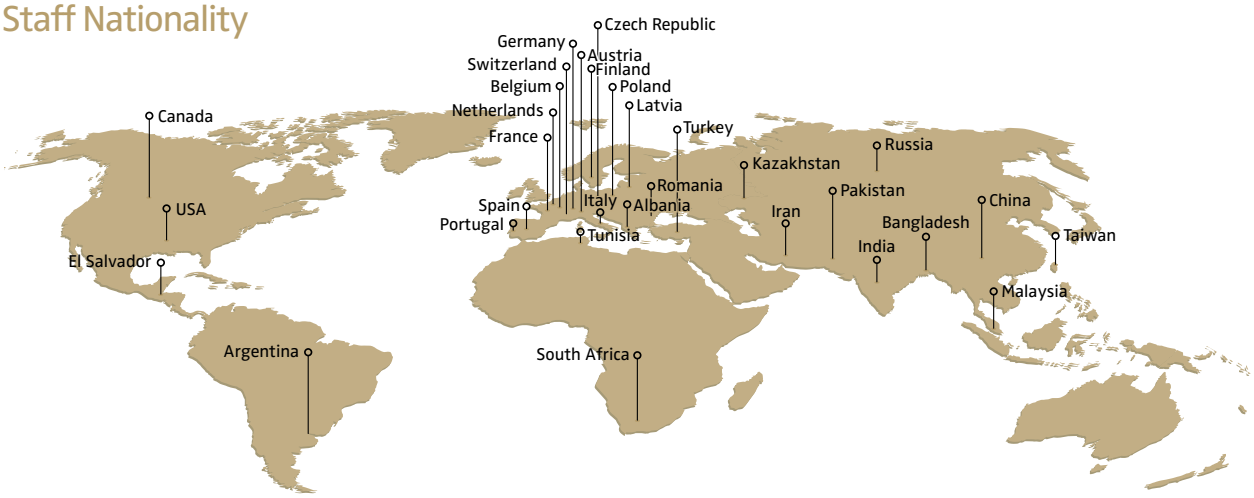
Visualizing and Targeting Cancer Stress

FACTS & FIGURES

Development of funds and human resources since 2005

Since Prof. Bernd Pichler became head of the newly founded laboratory in 2005, it has developed from a small laboratory into a leading international facility for imaging science. In 2008, the division of Radiopharmacy was merged with the Laboratory for Preclinical Imaging and Imaging Technology. Our success is demonstrated by a steady increase in the number of publications, the quality of the journals in which they are published, the funds we have raised and the growth and development of our personnel.

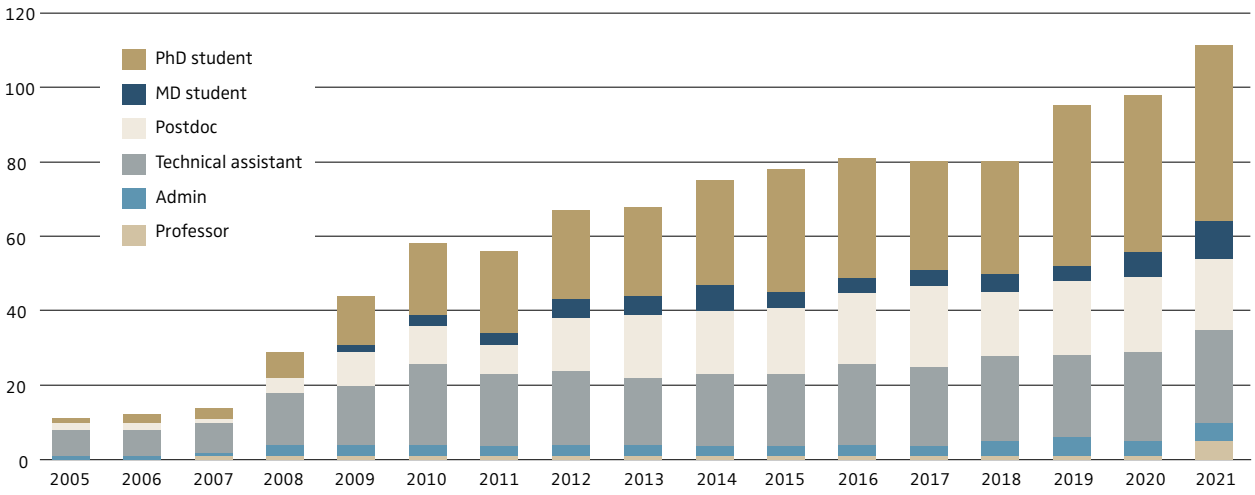
Staff Nationality



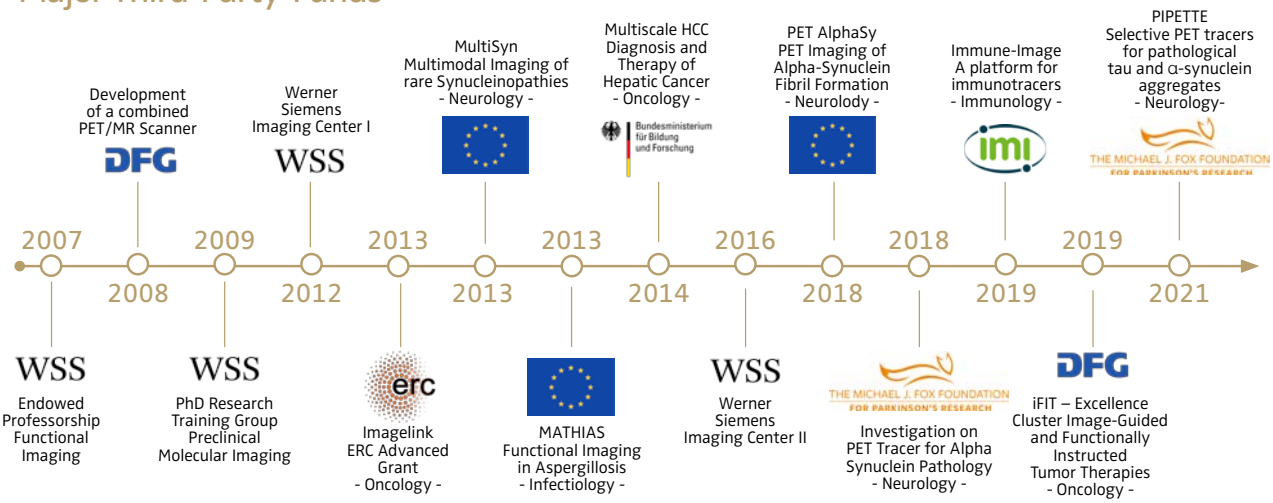
Previous Scientific Institution of Group Members

Albania: University of Tirana
Austria: Medical University of Vienna
Australia: University of Queensland
Bangladesh: National University of Bangladesh
Belgium: Free University of Brussels – ULB, Catholic University of Louvain
Canada: McGill University, University of British Columbia
Denmark: University of Copenhagen
El Salvador: University of El Salvador
France: University of Bordeaux, Université de Lille Droit et Santé, Université de Reims Champagne-Ardenne
Germany: Aachen University of Applied Sciences, Charité – Universitätsmedizin Berlin, University of Bonn, University of Erlangen–Nuremberg, Hochschule Furtwangen University, German Cancer Research Center, Karlsruhe Institute of Technology, University of Konstanz, Otto von Guericke University Magdeburg, University of Marburg, Munich University of Applied Sciences, University of Regensburg, University of Stuttgart, University of Hohenheim, University of Tübingen, University of Ulm, University of Würzburg)
Iran: Amirkabir University of Technology, University of Tehran
Ireland: University College Dublin
Italy: Polytechnic University of Milan, Vita-Salute San Raffaele University, University of Naples Federico II, University of Pavia, University of Turin
Netherlands: University of Twente
Pakistan: National University of Computer and Emerging Sciences
Poland: Poznan University of Medical Sciences
Russia: Mendeleev University of Chemical Technology of Russia
Switzerland: Swiss Federal Institute of Technology in Zurich
Spain: University of Granada, University of Valencia
South Arfica: University of Cape Town
Taiwan: National Tsing Hua University
UK: University of Birmingham, University of Cambridge, University College London
USA: Johns Hopkins University, NIH Clinical Center, Broad Institute of MIT and Harvard, University of California, Davis, University of Texas MD Anderson Cancer Center, University of California, Los Angeles, University of Dallas, Midwestern State University

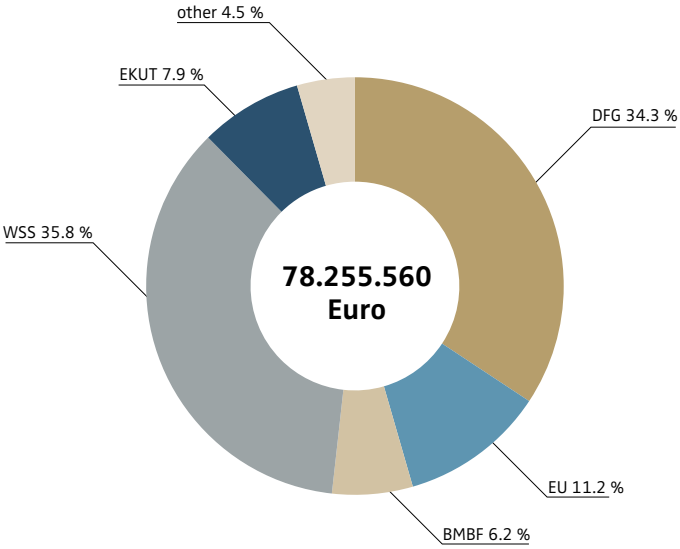
Personnel Development



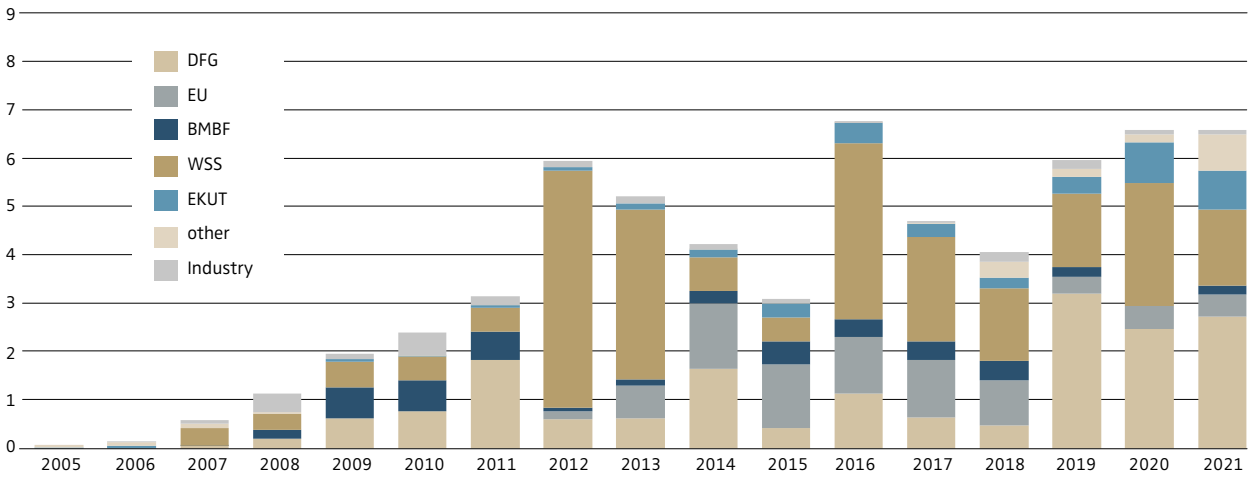
Major Third-Party-Funds



Fund Sources



Budget Development in Mio €



Legend: Deutsche Forschungsgemeinschaft (DFG - German Research Foundation); European Union (EU), Bundesministerium für Bildung und Forschung (BMBF - Federal Ministry of Education and Research); Werner Siemens Stiftung (WSS – Werner Siemens Foundation); Eberhard Karls University Tübingen (EKUT)

03

COOPERATIONS

COOPERATION WITH INDUSTRY

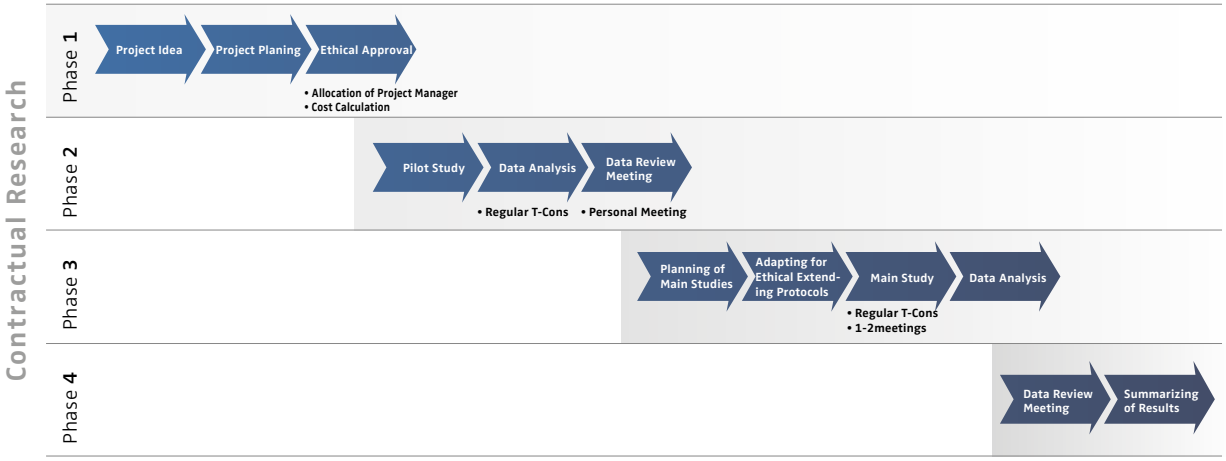
Our department is an academic facility with more than fifteen years of experience in contractual research with pharmaceutical companies. The benefits from this cooperation are threefold:

- Close links with pharmaceutical companies broaden the spectrum of our scientific studies by allowing the implementation of new research strategies and by providing access to novel drugs.
- Our researchers are exposed to the scientific work environment at companies, which is an important experience for fostering their professional careers.
- Contractual research can lead to joint publications or, if the sponsor requires confidentiality, financial support, providing greater flexibility for our research by maintaining a strong laboratory infrastructure.

The Department of Preclinical Imaging and Radiopharmacy is hosted within the Department of Radiology at the University Hospital Tübingen. Thus:

- The results from basic research can quickly be transferred to clinical departments for clinical validation.
- An early clinical trial unit (ECTU) for medical trial volunteers allows close supervision of study parameters.
- The Department of Radiology maintains a clinical trial office dedicated to supporting imaging phase I-III clinical imaging studies.
- The laboratory is backed up by the University Hospital's professional administration.

Currently, our laboratory maintains research collaborations with more than eleven major national and international pharmaceutical and technological companies.



ACADEMIC COOPERATION

UNIVERSITY HOSPITAL TÜBINGEN

- Department of Conservative Dentistry, Periodontology and Endodontology
- Center for Women's Health, Research Centre for Women's Health
- Department of Cellular Neurology
- Department of Neurology with Neurovascular Medicine and Neuro-Oncology
- Department of Neurodegenerative Diseases
- Department of General Psychiatry and Psychotherapy
- Department of Diagnostic and Interventional Neuroradiology
- Department of Diagnostic and Interventional Radiology
- Department of Nuclear Medicine and Clinical Molecular Imaging
- Department of Anesthesiology and Intensive Care Medicine
- Department of Dermatology
- Department of Experimental and Clinical Pharmacology and Toxicology
- Department of Internal Medicine I - Gastroenterology, Hepatology and Infectious Diseases
- Department Internal Medicine III - Cardiology and Circulatory Disorders
- Department Internal Medicine VIII - Medical Oncology & Pneumology
- Department of Otolaryngology, Head and Neck Surgery
- Department of Radiation Oncology
- Department of Urology, Division Regenerative Urology
- Institute of Medical Genetics and Applied Genomics
- Institute for Medical Psychology and Behavioural Neurobiology
- Department of General Paediatrics, Oncology/Haematology
- Department of Neuropaediatrics, Developmental Neurology, Social Paediatrics

UNIVERSITY OF TÜBINGEN

- Department of Chemistry, Institute of Organic Chemistry
- Department of Pharmacy and Biochemistry, Institute of Pharmaceutical Sciences
- Department of Physics, Institute for Astronomy and Astrophysics
- Interfaculty Institute for Cell Biology, Department of Immunology
- Interfaculty Institute for Cell Biology, Department of Molecular Biology
- Interfaculty Institute of Biochemistry, Division Lymphocyte Activation
- Natural and Medical Sciences Institute, Department Pharma and Biotech

GERMANY

- Max Planck Institute for Biological Cybernetics, Tübingen
- Max Planck Institute for Intelligent Systems, Tübingen & Stuttgart
- RWTH Aachen University, Aachen
- Charité - Berlin University of Medicine, Berlin
- University of Bonn, Bonn
- University Hospital Essen, Essen
- Goethe-University Frankfurt, Frankfurt
- Albert Ludwig University of Freiburg, Freiburg
- University Medical Center Göttingen, Göttingen
- Max Planck Institute for Biophysical Chemistry, Göttingen
- Hannover Medical School, Hannover
- German Cancer Research Center, Heidelberg
- Ruprecht-Karls-University Heidelberg, Heidelberg
- Karlsruhe Institute of Technology, Karlsruhe
- BG Trauma Center Ludwigshafen, Ludwigshafen
- University Medical Center Mainz, Mainz
- Johannes Gutenberg University Mainz, Mainz
- Philipps University of Marburg, Marburg
- LMU Munich, München
- Technical University of Munich, München
- University of Münster, Münster
- Julius Maximilian University of Würzburg, Würzburg

EUROPE

- Medical University of Vienna, Vienna, Austria
- Medical University of Innsbruck, Innsbruck, Austria
- University of Antwerp, Antwerp, Belgium
- Catholic University of Leuven, Leuven, Belgium
- University of Liège, Liège, Belgium
- University of Copenhagen, Copenhagen, Denmark
- University of Paris-Saclay, Orsay, France
- University of Reims Champagne-Ardenne, Reims, France
- VU University Medical Center, Amsterdam, Netherlands
- University Medical Center Groningen, Groningen, Netherlands
- Leiden University Medical Center, Leiden, Netherlands
- University of Leiden, Leiden, Netherlands
- Radboud University Medical Center, Nijemegen, Netherlands
- Erasmus University Medical Center, Rotterdam, Netherlands
- Norwegian University of Life Sciences, Ås, Norway
- University of Coimbra, Coimbra, Portugal
- Lund University, Lund, Sweden
- Uppsala University, Uppsala, Sweden
- Swiss Federal Institute of Technology (ETH) Zürich, Zürich, Switzerland
- University of Zürich, Zürich, Switzerland
- University of Cambridge, Cambridge, UK
- University of Exeter, Exeter, UK
- King's College London, London, UK

NORTH AMERICA

- University of British Columbia, Vancouver, Canada
- The Johns Hopkins University, Baltimore, Maryland, USA
- Harvard Medical School, Boston, Massachusetts, USA
- University of Texas Southwestern Medical Center, Dallas, Texas, USA
- University of Texas MD Anderson Cancer Center, Houston, Texas, USA
- New Jersey Institute of Technology, Newark, New Jersey, USA
- Memorial Sloan Kettering Cancer Center, New York City, New York, USA
- Stanford University, Stanford, California, USA

AUSTRALIA

- The University of Queensland, Brisbane, Australia
- Queensland Institute of Medical Research, Brisbane, Australia

04

DEPARTMENT OF PRECLINICAL IMAGING AND RADIOPHARMACY

DEPARTMENT ORGANIZATION

Director, Professors, Administration and Research Group Leaders

Department of Preclinical Imaging and Radiopharmacy

W2 Professorship	W2 Professorship	Chair of Department	W3 Professorship	Extracurricular Professorship
Prof. Dr. Kristina Herfert	Prof. Dr. Bettina Weigelin	Prof. Dr. Bernd Pichler Deputy: Dr. Julia Mannheim	Prof. Dr. André Martins	Apl. Prof. Dr. Gerald Reischl

Office and Administration

Personal Assistant	Head of Office, Human Resources	Fund Management	Scientific Coordination	Leading Technologist	Animal Care, Teaching, Safety	Industrial Cooperation	Radiopharmacy Controlling, IT
Ines Herbon	Dr. Rebecca Rock	Dr. Andreas Dieterich	Dr. Neele Hübner	Funda Cay	Dr. Carsten Calaminus	Dr. Marcel Krüger	Hans Jörg Rahm

Werner Siemens Imaging Center

Oncology	Oncology - Targeted Radionuclide Therapy	Advanced Pre-clinical Metabolic Imaging and Cell Engineering	Immunology & Inflammation	Preclinical Imaging of the Immune System	Infection & Inflammation	Functional and Metabolic Brain Imaging	Multiparametric Data Analysis & Mining	Imaging Detector Physics	MR & Multimodal Imaging Science	PET & Multimodal Imaging Science	Metabolomics & Systems Medicine	Imaging Probe Development	Radio-pharmacy
Prof. Dr. Bernd Pichler	Dr. Marcel Krüger	Prof. Dr. André Martins	Dr. Manfred Kneilling	Prof. Dr. Bettina Weigelin	Dr. Nicolas Bézière	Prof. Dr. Kristina Herfert	Prof. Dr. Bernd Pichler	Dr. Fabian Schmidt	Dr. Andreas Schmid	Dr. Julia Mannheim	Dr. Christoph Trautwein	Dr. Andreas Maurer	Apl. Prof. Dr. Gerald Reischl

Radiopharmacy

RESEARCH HIGHLIGHT
FROM THE IDEA TO THE CLINICS

Senescence is a response to cellular stress that is characterized by durable cell cycle arrest and distinct metabolic changes. It is a complex state that, depending on the circumstance, plays anti-oncogenic and pro-oncogenic roles. Many cancer therapies are known to induce senescence, and new compounds that specifically eliminate senescent tumor cells are under preclinical and clinical investigation. The development of a diagnostic tool to detect senescent cells in cancer patients *in vivo* is of great interest to optimize and guide the development of novel, next-generation tumor therapies. Most importantly, targeting senescent cells might provide completely new strategies to treat solid tumors and overcome therapy resistance. Thus, our work will very likely provide new hope for cancer patients.

TRACER DEVELOPMENT

Due to the need to image this important cell-state, we have worked with our clinical partners to develop a tracer that allows us to image senescence noninvasively in animals and now in patients. Senescence-associated β-galactosidase (SABG) is currently considered to be the gold-standard biomarker of senescence, and its expression is highly upregulated in senescent cells. Our tracer [^{18}F]FPyGal is a radiolabeled substrate of this important enzyme. Upon intracellular cleavage of the tracer by SABG, the radiolabeled metabolite is retained by senescent cells, allowing us to monitor senescence *in vivo* using PET. [^{18}F]FPyGal was synthesized and radiolabeled in the Werner Siemens Imaging facilities as part of Dr. Jonathan Cotton's PhD work. The tracer was subjected to chemical, *in vitro* and preclinical assessment by several PhD and medical students, whose hard work helped to validate its effectiveness toward imaging senescence. After seeing the tracer's preclinical success, we had high hopes for its clinical implementation and commissioned an external toxicity test in which the compound was actually proven to be very safe. We then had a pharmaceutical company produce a high-quality precursor in GMP quality and, under the direction of Apl. Prof. Dr. Gerald Reischl and Dr. Gabriele Kienzle, established a GMP-compliant radiosynthesis method so that we could use the tracer safely in humans. The first applications of the tracer in patients were performed under the umbrella of compassionate use by Prof.

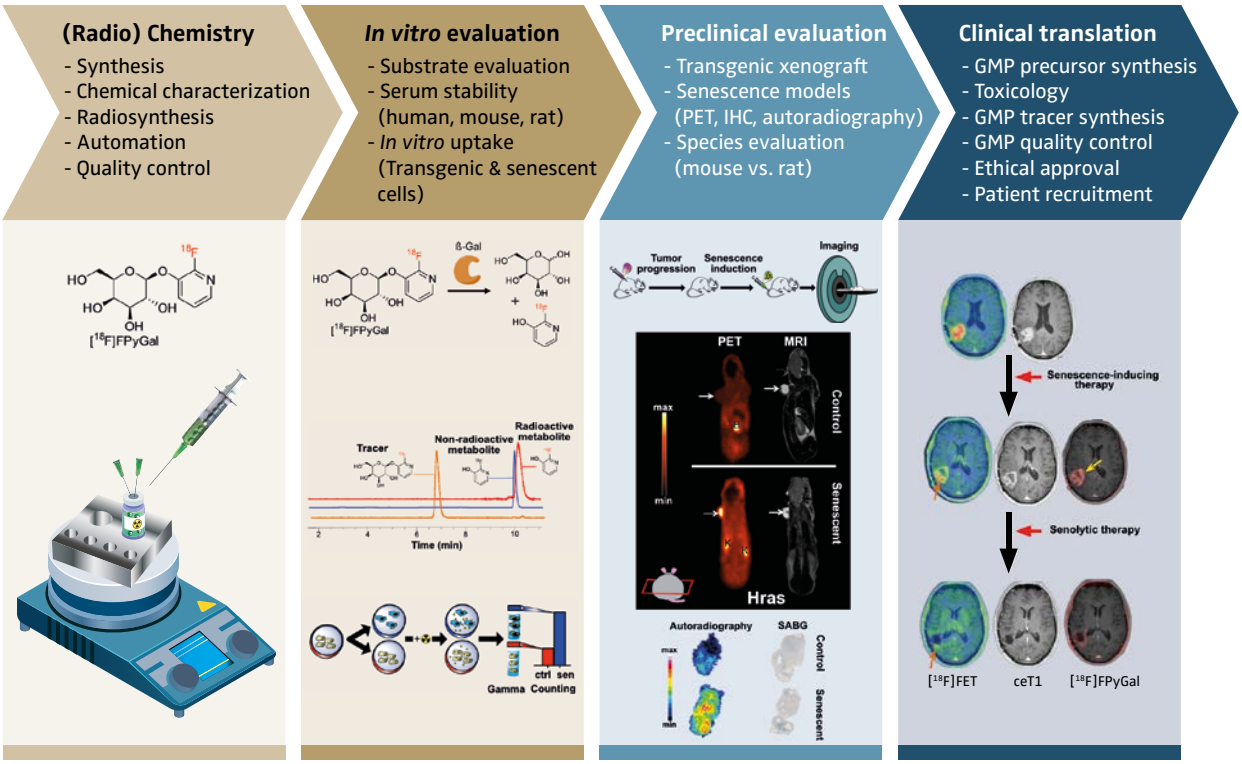


Figure 1: The tracer pipeline begins with chemistry and radiochemistry. Once synthesized and radiolabeled, the tracers are then evaluated *in vitro* to confirm that they are suitable substrates of their target enzymes. We also evaluate the tracer uptake in cells. The best tracer candidates are used for preclinical imaging experiments, in which tracer uptake can be visualized and compared between senescent and nonsenescent tumors. Finally, the best tracers can be clinically translated. In this image, we can see exciting imaging results from a human glioma patient who was scanned with FET (a tracer for tumor proliferation) and FPyGal (a tracer for senescence). We can see distinct and complementary uptake profiles of the two tracers after the patient received senescence inducing therapy. More excitingly, we see that after the patient received senolytic therapy, there was notably reduced uptake of both tracers. Thus, the PET images revealed by the new tracer directly impact tumor treatment and enable image-guided tumor therapy.

A large and interdisciplinary team of radiochemists, chemists, biochemists, biologists and physicians is required to translate a new diagnostic compound like the [^{18}F]FPyGal from the laboratory bench to the patient. The project has been started seven years ago which demonstrates the labor intensive steps of a diagnostic and drug development pipeline. Left to right: Prof. Dr. Christian la Fougère, PD Dr. Brigitte Gückel, Dr. Gabriele Kienzle, Apl. Prof. Dr. Gerald Reischl, Maren Pritzkow, Prof. Dr. Bernd Pichler, Dr. Francisco José Reche Pérez, Dr. Marcel Krüger, Dr. Jonathan Cotton, Benyuan Zhou. Missing: Prof. Dr. Lars Zender, Dr. Johannes Schwenck, MD, PhD.

Dr. Christian la Fougère (Department of Nuclear Medicine and Clinical Molecular Imaging) and Prof. Dr. Lars Zender (Department Internal Medicine VIII), and yielded promising initial results. The subsequent planning and execution of a Phase 1 clinical trial was coordinated by PD Dr. Brigitte Gückel and this imaging study was very successful. We are now in the process of planning our phase 2 clinical trial, in which we will be able to test the tracer in patients.

TRACER PIPELINE

Using what we have learned from the development and evaluation of [^{18}F]FPyGal, we have established a senescence tracer evaluation pipeline (see Figure 1), which we are currently using to rapidly investigate second-generation senescence tracers. Tracer candidates and reference compounds are conceived, designed and synthesized in our state-of-the-art chemistry labs. After full chemical characterization, work to radiolabel a precursor can begin. This usually starts with a 'manual synthesis', wherein radiolabeling is performed manually using fluoride that is provided by our radiopharmacy. The radiosynthesis is then optimized and automated so that the tracer candidate can be safely and reliably prepared. Afterwards we evaluate the biological properties. The radiolabeled SABG substrates are first incubated with β-galactosidase to determine if they are metabolized and produce the expected radio-metabolite. We can then apply the tracer candidate to cells (transgenic β-galactosidase-overexpressing or senescent cells and their respective controls) and measure how much of the radioactivity is taken up. This gives us a good first impression of the *in vivo* performance. The biological stability is evaluated by incubating the tracer candidates in serum (human, mouse, rat) for up to 4 hours and using radio-HPLC to screen for unexpected metabolites. After *in vitro* evaluation, we are ready to apply the tracer candidate to various tumor models. For a typical *in vivo* experiment, mice were implanted with tumors and divided into two groups. One group receives senescence-promoting therapy, and the other group served as the control group. After injecting the tracer candidate and scanning the mice, we can evaluate the PET images to determine whether we can differentiate between senescent and nonsenescent (control) tumors. To validate our *in vivo* findings, we rely on *ex vivo* histology and SABG staining to confirm that the signal we observe in the tumor PET images correlates to senescence (or proliferating tumors). Once a tracer has been thoroughly evaluated in preclinical models, we can begin the process of clinical translation. This involves synthesizing GMP-compliant precursors and performing comprehensive toxicological studies. GMP tracer synthesis and GMP quality control were established by our radiopharmacy, which has extensive experience in this area. Clinical evaluation will be performed in a full clinical trial conducted by our clinical partners.

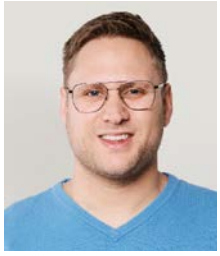
CLINICAL STUDY HIGHLIGHTS

Due to the promising preclinical results we applied [^{18}F]FPyGal in a glioblastoma patient and correlated [^{18}F]FPyGal uptake with [^{18}F]FET uptake, which indicates tumor cell proliferation. Interestingly, we identified regions with high [^{18}F]FET and low [^{18}F]FPyGal uptake and vice versa, supporting our theory that [^{18}F]FPyGal indicates senescent tissue and can be used for the guidance of therapy (Figure 1). A Phase I/II clinical has been initiated.

PATENTS

The Medical Faculty of the University of Tübingen has filed two patents for senescence tracers. One of these patents has been published (Senescence tracers; EP2890981A1), and one is pending (Radiolabeled beta-galactosidase substrate for PET imaging of senescence, WO2018153966A1).

Prof. Dr. Bernd Pichler
Group Leader Oncology
+49-7071-29-83427
bernd.pichler@med.
uni-tuebingen.de



Dr. Jonathan Cotton
+49-7071-29-80566
jonathan.cotton@med.
uni-tuebingen.de

ONCOLOGY

Our imaging modalities allow us to noninvasively and longitudinally reveal a wide variety of physiological processes in tumors and premalignant tissues.

Therefore, our scientific work includes preclinical studies focusing on immuno-, molecular targeted and combinatorial therapies as well as the improvement and evaluation of novel diagnostics. Additionally, basic research in the field of metastasis, tumor microenvironment and senescence is ongoing in our laboratory. Our experienced radiopharmacy enables us to employ a wide range of conventional PET tracers, such as FDG, FLT and many more, as well as novel tracers, such as ^{64}Cu- or ^{89}Zr-labeled antibodies, nanobodies and peptides. For therapeutic approaches, we have well-developed workflows to handle large amounts of ^{177}Lu.

Our group has great experience with a wide range of different tumor models, ranging from classical subcutaneous tumor xenograft models to orthotopic brain tumor models, patient-derived, endogenous, chemical or diet-induced models and transplanted tumor models.

In an increasingly interdisciplinary environment, we are not restricted to imaging modalities such as PET, MRT (including advanced MRI techniques such as apparent diffusion coefficient (ADC) maps or MR spectroscopy), CT, optical imaging (OI) or single-photon emission computed tomography (SPECT), but we also attempt to link the imaging data to molecular processes. Our in-house nuclear magnetic resonance (NMR) spectrometer enables us to analyze the metabolome of tumors, and proteomic, genomic and transcriptomic analysis is performed in close collaboration with our partners within the university.

TARGETED RADIONUCLIDE THERAPY (TRT)

TRT is an emerging cancer treatment that relies on the fact that many tumor cells express specific antigens that are either only expressed by malignant cells or whose expression is strongly upregulated in tumor tissues compared to healthy tissues. A variety of molecules, ranging from small molecules to peptides, antibody fragments and full antibodies, have been developed that specifically bind to these tumor antigens. For use in TRT, these molecules need to be labeled with radioactive isotopes emitting alpha or beta radiation and can then act as carriers for the respective therapeutic isotopes. Ideally, molecules that are not bound in the tumor will be rapidly cleared from the bloodstream by the kidneys and are subsequently excreted via the bladder. In this way, the radioactive isotope is mainly enriched in the tumor tissue, while healthy tissues harbor only limited amounts of radioactivity.

The radioactive decay of isotopes leads to the emission of high-energy particles that interact with the surrounding matter and generate oxygen radicals that chemically modify macromolecules, such as proteins and DNA. This can lead to single- (SSB) or double-strand breaks (DSB), which must be repaired by the affected cell. If the amount of DNA damage exceeds a certain threshold, the cell is inevitably driven into cell death. Various approaches are currently being pursued to improve the effectiveness of TRT. In the past, we tested a novel targeting molecule that shows higher affinity for its target than an established one (Figure 1). Thus, it is possible to achieve a higher tumor radiation dose with the same injection dose and therefore comparable side effects. Unfortunately, the development of novel targeting molecules is time-consuming and might not always be possible.

In the future, we also want to improve the efficacy of TRT by combining it with therapies that target the DNA damage response (DDR). This relies on the fact that TRT causes radiation-induced DNA damage, specifically in tumors. As a result, functional DNA repair mechanisms become of crucial importance for tumor cells. If these mechanisms are specifically restricted by inhibitors, it can be expected that the effectiveness of TRT will be significantly increased. Furthermore, we want to extensively analyze the tumor response after TRT toward senescence.

IMAGE-GUIDED TISSUE EXTRACTION

Phenotypic heterogeneity is commonly observed specifically in tumors. By applying multimodal imaging technologies, tissue heterogeneity can be revealed noninvasively *in vivo* on a macroscopic scale. Methods such as multiomics, immunohistochemistry or histology comprehensively characterize these heterogeneities and detail them down to a cellular scale. However, all of these approaches are *ex vivo* methods and require tissue extraction. Complementary *in vivo* and *ex vivo* information would provide substantial potential to better characterize a disease. To achieve this, spatially accurate coregistration of *ex vivo* and *in vivo* data by image-driven sampling, including fast sample preparation, is required. We have developed a unique image-guided milling machine (IGMM) that enables us to precisely extract tissue samples from mice (Figure 2a-c). With this machine, it is possible to define volumes of interest on tomographic images and to extract these volumes with a spatial accuracy better than 0.25 mm (Figure 2d). During the process, samples remain cooled and can be used for metabolic or proteomic analysis (Figure 2e) (Disselhorst, Krüger et al., Proc Natl Acad Sci U S A. 2018;115(13)).

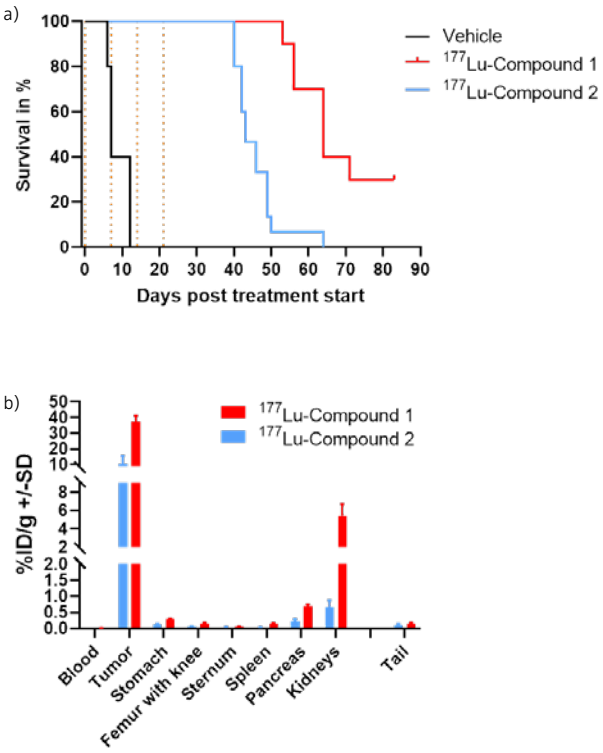


Figure 1: a) Survival of tumor-bearing mice treated 4 times with vehicle or equal doses of ^{177}Lu-Compound 1 or ^{177}Lu-Compound 2 with equal specific activities. Dotted lines indicate treatments. b) Biodistribution of ^{177}Lu-Compound 1 and ^{177}Lu-Compound 2 96 hours after the last treatment.

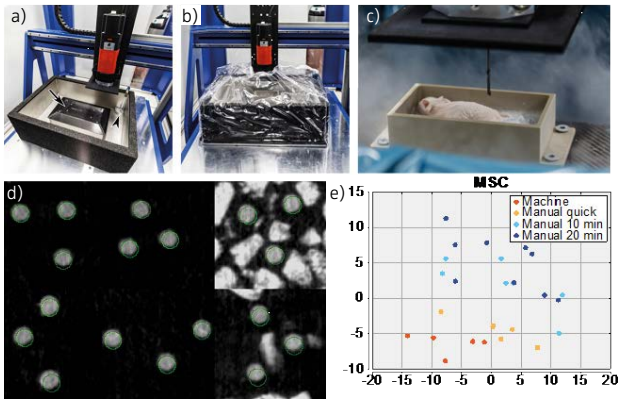


Figure 2: a) Overview of the IGMM showing the components of the milling machine, i.e., the liquid nitrogen bath and the inflow valve for gaseous nitrogen (arrowhead), both for cooling the samples and the attachment plate for the holders (arrow) to accurately fix the animal on the machine. b) The cover around the milling area is used to contain the cold nitrogen vapor. c) Close-up of a holder with an embedded, frozen mouse. Notice the nitrogen vapor surrounding the milling area and the milling bit shown at the top. d) CT images acquired postmilling from 6 mice with 3 regions each. The volumes of interest, as defined before milling, are shown in green. The gray circles show the real location of the cylinders after milling, determined by CT imaging. e) Effect of handling conditions: tissue samples were collected with the IGMM or manually at different time points after termination of the animal. The samples were subsequently analyzed by NMR, and a principal component analysis of the NMR spectra was performed. The proximity of the points indicates how similar the two acquired spectra are (Disselhorst, Krüger et al., Proc Natl Acad Sci U S A. 2018;115(13)).

Prof. Dr. Bernd Pichler
Head of Oncology Research
+49-7071-29-83427
bernd.pichler@
med.uni-tuebingen.de

Dr. Marcel Krüger
Group Leader Oncology - Targeted Radionuclide Therapy
+49-7071-29-82972
marcel.krueger@
med.uni-tuebingen.de

IMMUNOLOGY & INFLAMMATION

Inflammation is caused by immune reactions induced by pathogens, such as bacteria, viruses, fungi and parasites; by degenerated malignant or foreign cells; and by chemical or physical stimuli, including ionizing radiation, burns, frostbite or trauma. An inflammatory immune response protects the host by eliminating pathogens, removing necrotic cells and initiating tissue repair and wound healing. Inflammation is normally self-limiting, but excessive and destructive immune responses that target the body itself can lead to autoimmune diseases, such as rheumatoid arthritis (RA) or multiple sclerosis. However, inflammation is involved in many more human pathologies, including atherosclerosis, carcinogenesis, cancer immunity, Alzheimer's disease, allergic reactions and stroke, but its therapeutic modulation is still an ongoing challenge, not least due to limited differential diagnostic possibilities.

Thus, the aim of our research is to gain deeper insights into the pathophysiology of different inflammatory immune responses by developing advanced imaging modalities such as PET/CT, PET/MRI, OI and imaging probes specific for inflammatory processes. This will allow us to noninvasively follow disease progression and to monitor successful anti-inflammatory treatments *in vivo*, which will allow the development of patient-individualized therapies.

Basic experimental models of inflammatory diseases, such as the T cell-mediated contact hypersensitivity reactions of the skin, are helping us understand and visualize the dynamics of pro-inflammatory/pro-angiogenic mediators, reactive oxygen and nitrogen species (ROS/RNS; Figure 1) and hypoxia (Figure 2) and to follow the homing patterns of different inflammatory cells (e.g., T cells; Figure 3 left). In particular, the detection of early stages of tissue-destructive inflammatory immune responses, such as in rheumatoid arthritis, is of great importance to enable early treatment and prevent joint destruction and the resulting disability.

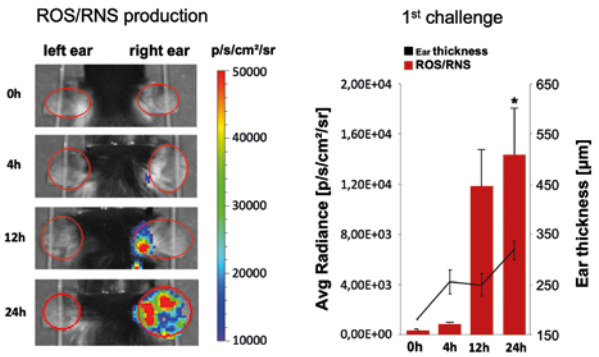


Figure 1: Noninvasive *in vivo* optical imaging measurement of the temporal dynamics of ROS/RNS production in ears inflamed by an acute TNCB-specific contact hypersensitivity reaction using L-012, a ROS/RNS-sensitive chemiluminescence optical imaging compound. Left: *in vivo* L-012 optical imaging images; Right: Quantification of RNS/ROS production and changes in ear thickness before and 4, 12 and 24 hours after the elicitation of acute TNCB-specific contact hypersensitivity reaction. (Schwenck et al., Mol Imaging Biol. 2020;22(3)).

CANCER AND THE IMMUNE SYSTEM

The immune system and its cells (T cells, natural killer cells, macrophages, neutrophils) and soluble mediators (IFN-β, TNF, IL-2, VEGF, MMPs) have different effects on cancer cells and can promote either cancer rejection or cancer progression.

Cancer cells can escape the endogenous anti-tumoral immune response by expressing programmed death ligand-1 (PD-L1), which binds to its ligand programmed death-1 (PD-1) that is expressed predominantly by T cells. PD-L1/PD-1 signaling drives tumor antigen-specific 'therapeutic' T cells to undergo apoptosis. Thus, targeting the PD-L1/PD-1 pathway by specific monoclonal antibodies (mAbs) against PD-1 or PD-L1 has been proven to be a successful strategy for the so-called immune checkpoint inhibitor treatment of metastatic melanoma (anti-PD-1 mAbs), Merkel cell carcinoma (anti-PD-L1 mAbs), lung cancer and other malignancies.

Consequently, we used our expertise in inflammation and immunology research to explore carcinogenesis and cancer immunity by *in vivo* imaging. The main interests of our cancer immunotherapy (CIT) research are

1. to promote the early identification of immune therapy responders by holistic noninvasive *in vivo* imaging of primary and secondary lymphatic organs (Figure 4) and by revealing activated T cells (Figure 3 right) in experimental mice and tumor patients in a translational manner;
2. to reveal the physiology of systemic cancer immune responses by following the temporal dynamics of T cells and other leucocytes as well as to follow their migration, homing and activation noninvasively *in vivo* in experimental mice and cancer patients; and
3. to develop new combined immunotherapies for advanced solid cancers by targeting tumor antigen-specific T cells and different immune modulating agents, such as immune checkpoint inhibitors (CTLA-4, PD-1, PDL-1, PDL-2, LAG-3, TIM-3), whole body/fractionated radiation, oncolytic viruses, and modulation of the pH or microbiome.

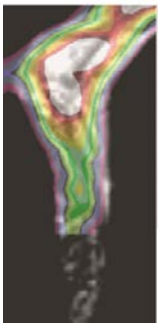


Figure 2: Noninvasive *in vivo* measurement of tissue hypoxia in experimental arthritis. Multimodal [^{18}F]FAZA PET/MR imaging reveals strongly pronounced [^{18}F]FAZA uptake in an arthritic ankle of an experimental mouse with GPI serum-induced arthritis, six days after the onset of arthritis (Fuchs et al., J Nucl Med. 2017;58(5)).

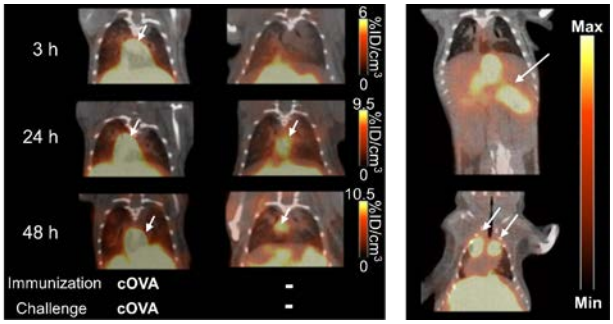


Figure 3: Noninvasive T cell tracking in lung inflammation and cancer. Left: PET/CT measurements of the trafficking of [^{64}Cu]Cu-DOTA chicken ovalbumin (cOVA) T cell receptor antibody-labeled cOVA T cells 3, 24 and 48 h after intraperitoneal injection into diseased mice with chicken cOVA-specific lung inflammation (left) and control mice (right). PET/CT images reveal enhanced homing of OVA-Th1 cells to the pulmonary lymph nodes in OVA-immunized and OVA-challenged mice. Right: PET/CT images of [^{64}Cu]Cu-DOTA-Thy1.2 mAb-labeled tumor antigen-specific T cells homing to the pancreatic area (upper image) and to the intraperitoneal administration-specific homing sites, the perithymic lymph nodes (arrows; lower image) and the spleen (arrow, upper image) in RIP1-Tag2 mice, an endogenous mouse model of multistep pancreatic cancer. (Griessinger et al., Proc Natl Acad Sci U S A. 2015;112(4)).

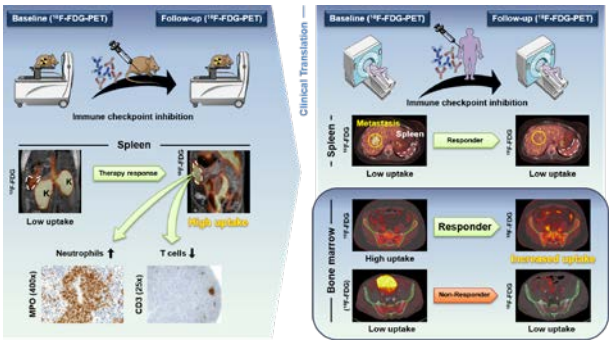
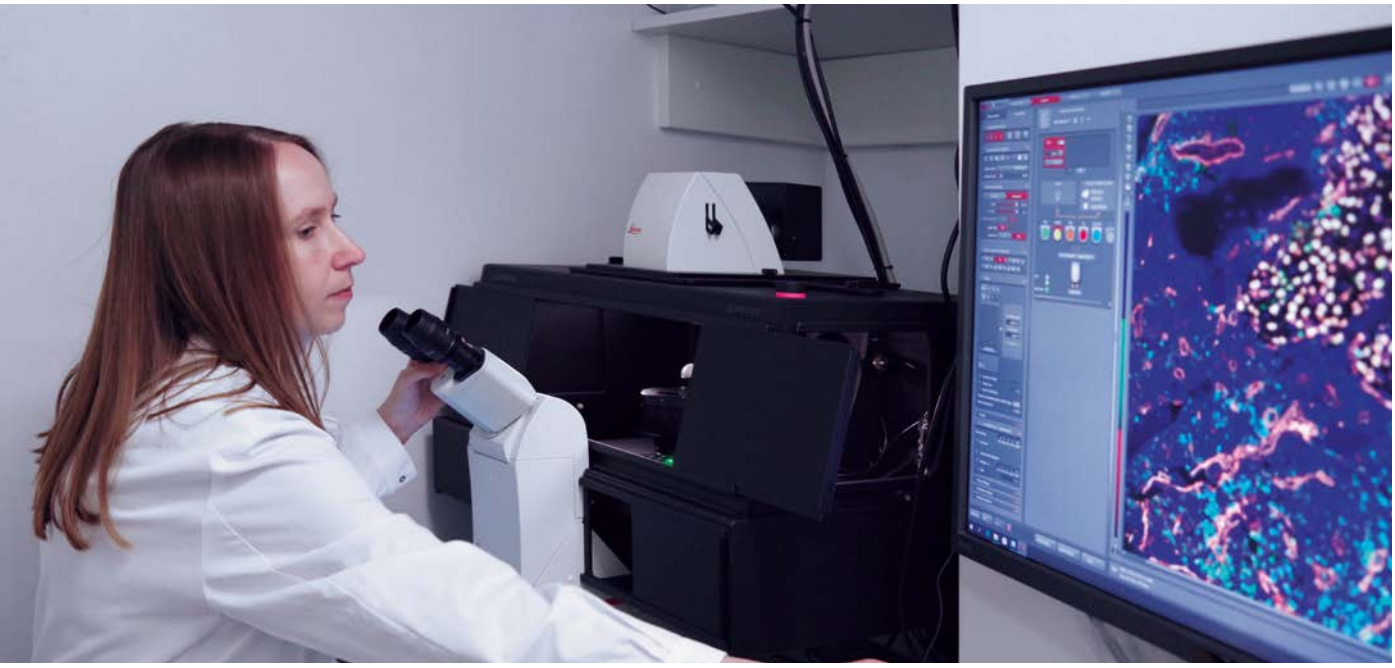


Figure 4: [^{18}F]FDG PET, a surrogate marker, is applicable for revealing successful checkpoint inhibitor therapy-based immune cell activation in the lymphatic organs of mice and patients with solid cancer. RIP1-Tag2 mice with advanced insular cell carcinomas treated with combination immunotherapy exhibited significantly increased [^{18}F]FDG uptake in the spleen compared to sham-treated mice. Immunohistochemistry of the spleens revealed a lower number of T cells and a higher number of neutrophils compared to those in the spleens of sham-treated mice. In addition, flow cytometry of the bone marrow showed enhanced activation of T cells following the treatment schemes that included checkpoint inhibitors. A retrospective analysis of clinical [^{18}F]FDG PET/CT scans revealed enhanced [^{18}F]FDG uptake in the spleens of some successfully CIT-treated patients with metastatic melanoma, but there were no significant differences between responders and nonresponders. The analysis of the bone marrow in clinical [^{18}F]FDG PET/CT scans with a computational segmentation tool revealed significantly higher baseline [^{18}F]FDG uptake in patients who responded to CIT than in nonresponders, and this relationship was independent of bone metastasis, even in the baseline scan.

Dr. Manfred Kneilling
Group Leader Immunology & Inflammation
+49-7071-29-86870
manfred.kneilling@med.uni-tuebingen.de



PRECLINICAL IMAGING OF THE IMMUNE SYSTEM

MULTISCALE MICROSCOPY OF CELLULAR IMMUNOTHERAPIES

Immunotherapy is an emerging first-line therapy for advanced cancer with the potential to achieve long-lasting regression and cure. Most solid tumors respond to immunotherapy to varying degrees, but the majority of patients experience resistance in tumor subregions followed by relapse. The main effector cells that mediate tumor control are cytotoxic T lymphocytes (CTLs), which kill cancer cells in a cell contact- and antigen-specific manner. However, although CTLs are observed to infiltrate tumors in patients, their ability to control tumor growth is often insufficient. Consequently, immunotherapeutic strategies aim to activate and expand tumor antigen-specific CTLs and further improve their ability to kill within the tumor. Current therapeutic approaches include inhibition of immune checkpoints to silence inhibitory receptors, vaccination with dendritic cells to stimulate endogenous antitumor immune activation and adoptive transfer of patient-derived, genetically modified T cells to enhance the number of tumor-specific cells with strong killing capacity. While each approach has demonstrated experimental and clinical success, no individual or combined strategy has achieved sufficient efficacy in a majority of patients.

MICROENVIRONMENT-CONTROLLED IMMUNE FUNCTION

The clinical success of adoptive T cell transfer in solid tumors appears to be limited by several immunosuppressive barriers imposed by the tumor microenvironment. Transferred CTLs are excluded from tumor lesions or become dysfunctional upon entry into the tumor, interaction with tumor cells is too short-lived to reach full cytotoxic potential, and suppressive immune cells directly or indirectly interfere with CTL activity. Furthermore, in the same patient, distinct microenvironments, such as those at the primary tumor site and metastatic lesions, differ in their response to therapy. In particular, bone metastases are typically resistant to immunotherapy despite successful immune infiltration of the primary lesion. The factors in the bone marrow that inhibit the efficiency of cell-based immunotherapies and the biomarkers that can be used to detect therapeutic failure in a patient at an early stage are only incompletely understood. In ongoing studies, we aim to correlate information from macroscopic imaging (PET/MRI) with cellular and molecular profiling to identify immunosuppressive signatures in bone that can serve as biomarkers for noninvasive therapy monitoring as well as target structures for new therapeutic approaches.

SYNERGY OF MACRO- AND MICROSCALE IMAGING

Experimental approaches that investigate the immunotherapy response in small animals are mostly based on static images (e.g., immunohistochemistry) or macroscopic imaging (e.g., bioluminescence, CT, PET, MRI). Although informative, these strategies lack sensitivity or temporal and spatial resolution to characterize dynamic and reciprocal interactions between tumor cells, the microenvironment and immune effector cells, which occur in defined tissue niches. Mechanistic, 3D and time-resolved insights into the positioning and function of single cells during therapy, their adjacent environment, and tumor-stroma interactions have been made possible by technological advances in intravital multiphoton microscopy (iMPM). Intravital microscopy is suitable for accessing niches that mediate tumor progression, including cell growth,

motility and invasion, remodeling of the stroma, neovessel anatomy and function, and regulation of molecular signaling events. Similarly, iMPM is capable of capturing all functionally relevant steps of adoptive T cell therapy, including the arrival of transferred cells in the lesion, early effector function and induction of tolerance and CTL exhaustion (Figure 1). Thus, microscopic imaging provides mechanistic insight at the single cell level and is essential for developing the bases for novel treatment approaches. Macroscopic imaging allows the monitoring of systemic immune effects and the early identification of therapy efficacy or potential side effects in patients. Ongoing research addresses how both imaging scales can be combined to deliver synergistic information about immune function during therapy (Figure 2).

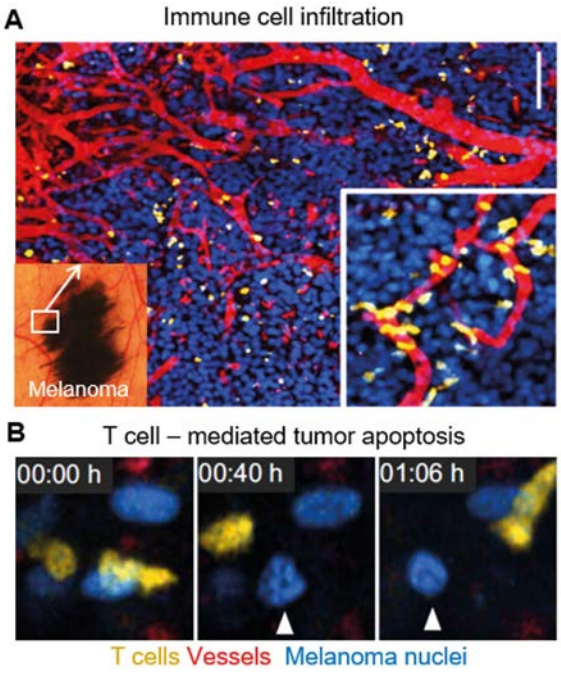


Figure 1: Intravital multiphoton microscopy allows the monitoring of immune cell function and tumor therapy response at the single-cell level (A). Visualization of essential steps during immunotherapy, such as immune cell arrival through blood vessels and infiltration and positioning of immune cells within the tumor, as well as single cell dynamics, such as immune cell proliferation and tumor immune cell contact followed by tumor cell apoptosis (B). Therefore, rate-limiting steps and tumor resistance niches can be identified, which provide rationales for novel treatment strategies and synergistic therapy combinations.

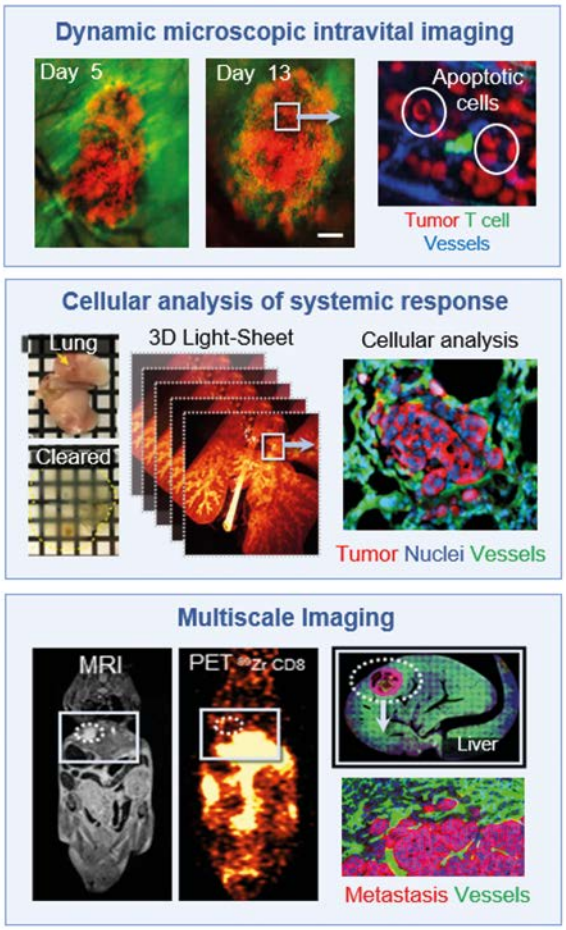


Figure 2: Microscopic imaging at subcellular resolution provides mechanistic insight into therapy success or failure at the single-cell and tissue levels and is essential for developing the bases of novel treatment approaches. Macroscopic imaging allows the monitoring of systemic immune effects and the early identification of therapy efficacy or potential side effects in patients. Ongoing research addresses how both imaging scales can be combined to deliver synergistic information on immune function during therapy.

Prof. Dr. Bettina Weigelin
Head of Preclinical Imaging of the Immune System
+49-7071-29-87511
bettina.weigelin@med.uni-tuebingen.de

ADVANCED PRECLINICAL METABOLIC IMAGING AND CELL ENGINEERING

Cell metabolism plays an essential role in understanding the uptake of nutrients and the oxidation of crucial substrates in tissues. Recently, many reports about metabolic imaging have emerged, as this approach allows the development of novel, imaging-guided, targeted therapies for cancer, diabetes, stroke, and liver failure. At the Werner Siemens Imaging Center, we address these biochemical/biomedical challenges using cutting-edge, state-of-the-art, and highly translatable metabolic imaging.

HYPERPOLARIZED METABOLISM & CANCER PROFILING

Metabolic cancer profiling is a crucial focus of our laboratory, particularly for understanding of resistance to cancer immunotherapy. We are uniquely positioned to detect and predict real-time responses to therapy by molecular and metabolic imaging. For instance, we have a clinical SpinLabTM DNP hyperpolarizer that is a unique piece of equipment in Germany used to perform metabolic imaging. We have gained a vast amount of experience with this innovative multimodality imaging system over the last few years. Now, we combine metabolic imaging with both PET and MRI (HyperPET) so that in a single 1-2 hour imaging session, we can study perfusion, hyperpolarized metabolism, cellularity and therapy response in solid tumors. We have also implemented an MRI technique that is able to map extracellular pH and lactate levels in tissues *in vivo* by chemical exchange saturation transfer (CEST) (Figure 1) (Zhang et al., J Am Chem Soc. 2017;139(48)). All these techniques are currently applied in the clinic. They offer unique information about metabolic activity in tissues.

METABOLIC CLUSTERING AND MODULATION OF THE TUMOR MICROENVIRONMENT

Understanding the tumor microenvironment, heterogeneity and metabolism will provide critical insights that will lead to symptomatic improvement of the diagnosis and to personalized cancer therapies. We aim to address the challenges associated with tumor heterogeneity and aggressiveness with novel hybrid PET/MRI sensors, state-of-the-art hybrid imaging, multiparametric data analysis and machine learning and a quantitative functional imaging sensing approach. Our expertise will provide strategies to understand and modulate - with inhibitors currently undergoing clinical trials - tumor heterogeneity, aggressiveness and malignancy. It will also create opportunities for the development of personalized treatments through specialized diagnostic methods capable of detecting relevant metabolic biomarkers (e.g., metabolites, pH, signaling metals, reactive oxygen species).

QUANTITATIVE FUNCTIONAL IMAGING OF METABOLIC SENSORS

The development of 'smart' and responsive multimodal sensors that detect events in the extracellular space offers the possibility of adding accurate molecular imaging information in addition to outstanding temporal and spatial resolution. Here, we combine expertise from several colleagues at the Radiochemistry group to design the next generation of functional quantitative imaging probes (e.g., hybrid PET/MRI sensors). We aim to develop sensors that detect changes in specific enzymatic activity, tem-

perature, metal ion concentrations, oxygen pressure and reactive species, pH and metabolites (Martins et al., J Am Chem Soc 2018;140(50); Anbu et al., Angew.Chem. Int. Ed. 2021;60(19)). The Department of Preclinical Imaging and Radiopharmacy has state-of-the-art instrumentation ideal for this research (hybrid PET/MRI, PET/CT, and SPECT/CT scanners), modern small animal imaging facilities, GMP-capable radiochemistry with fully equipped laboratories that enable clinical translation.

METAL METABOLISM FOR MEDICAL IMAGING

Disrupted metal homeostasis is associated with pathological conditions, such as dementia, cancer and inherited metabolic abnormalities. Intracellular pathways involving essential metals have been extensively studied. However, whole-body fluxes and transport between different compartments remain poorly understood. Recently we demonstrated in a collaborative effort with the Harvard Medical School and the UT Southwestern that zinc and copper play a critical role in identifying stages of malignancy in prostate cancer (PCa) by MRI and synchrotron radiation X-ray fluorescence (SRXRF) (Jordan et al., Inorg Chem. 2019 Oct 21;58(20)). Preliminary results from the Werner Siemens Imaging Center also suggest that copper, zinc and manganese play an essential role in triple-negative breast cancer, pancreatic cancers and hepatocellular carcinomas (HCC). Here, we aim to achieve the preclinical translation of metal metabolism using hybrid molecular imaging, personalized diagnostics and specialized methods developed in-house: PET/MRI, HyperPET, functional quantitative imaging, PET/MRI sensors.

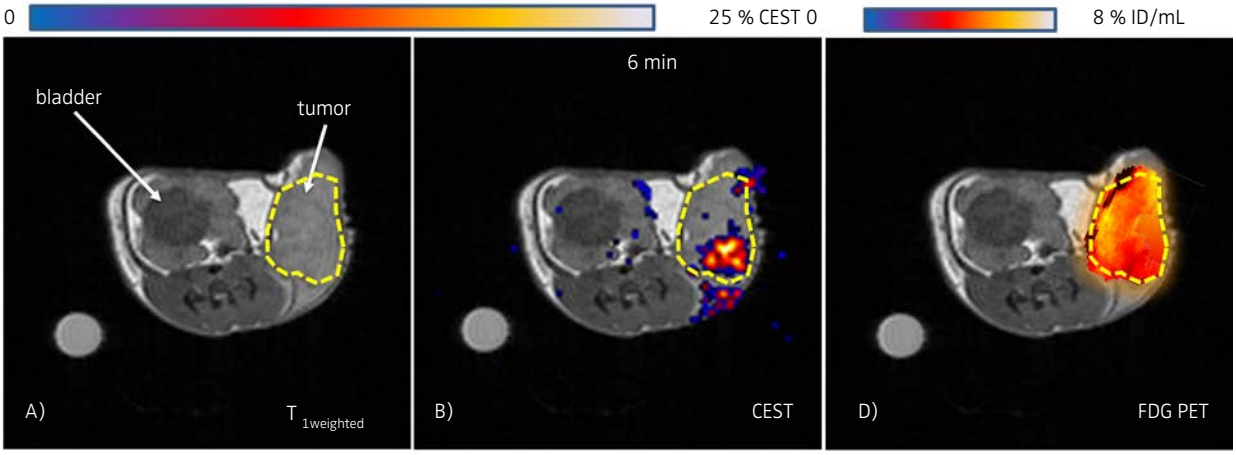


Figure 1: A) Axial view T1-weighted imaging and the respective B) *in vivo* CEST MR images of mice bearing a small cell lung cancer (SCLC) tumor in the lower flank after an i.v. injection of EuDO3A. D) Fused PET/MR image of the SCLC mouse model injected with 2-deoxy-2-[^{18}F]fluoro-D-glucose.

Unterstützt von / Supported by
Alexander von Humboldt Stiftung/Foundation

Our research has recently been recognized by the Alexander von Humboldt Foundation through the prestigious Sofja Kovalevskaja Award. The overall goal of the proposed research is to quantitatively access, predict and modulate metabolic tumor heterogeneity with noninvasive multifunctional/multimodal/multiparametric methods. We will use cutting-edge hybrid technology, machine learning, and highly translatable smart molecular imaging hybrid probes to identify novel metabolic features of cancer. This 5-year program will enable us to noninvasively functionally surveil, predict, select and image-guide tumor metabolic therapies with high precision. The work is also of critical importance for understanding the tumor response to immune checkpoint inhibitor therapies, cellular immunotherapies (e.g., CAR T cells or adoptive T cell transfer) and combinatorial therapies. Can therapeutic resistance be predicted and modulated by the tumor metabolic microenvironment?

Prof. Dr. André F. Martins
Head of Advanced Preclinical Metabolic Imaging and Cell Engineering
+49-7071-29-29-87487
andre.martins@med.uni-tuebingen.de

METABOLOMICS & SYSTEMS MEDICINE

METABOLOMICS

During the last few years, the field of biological sciences has become exponentially more interested in metabolomics since it represents the functional end-point of healthy or disease-related processes. As it not only depicts genetic predisposition but also environmental influences such as nutrition, exercise or medication, metabolomics offers great perspectives for diagnosis and treatment in medicine.

Therefore, as a highly technology-driven research institution, we have continuously upgraded our instrumental portfolio for metabolomics. The core of the analytical setup is a high-field 600 MHz nuclear magnetic resonance (NMR) spectrometer equipped with different probes for the analysis of human biofluids (5 mm $^{1}H/^{13}C/^{15}N$ probe), mass- or volume-limited murine samples (1.7 mm $^{1}H/^{13}C/^{31}P$ probe) and semisolid-like tissue (4 mm $^{1}H/^{13}C$ High Resolution Magic-Angle Spinning (HR-MAS) probe) (Figure 1a/b). This analytical instrumentation is located downstream of several semiautomated sample preparation systems (cryogenic tissue pulverizer, lyophilisator, ultrasonic sample extraction system, vacuum concentrator), allowing high throughput with maximum reproducibility.

Within our interdisciplinary imaging research facility, we are performing different projects in the realms of neurology, oncology, inflammation and more. From those projects, many kinds of murine samples are available for metabolomics analysis. For instance, intratumoral heterogeneity in breast cancer is a major cause of disease progression and therapy resistance. To enable a spatially accurate match of *in vivo* imaging with *ex vivo* metabolomics data, we used an image-guided milling machine and cross-matched biopsy metabolite data with imaging parameters (Figure 2a).

If in addition to tissue metabolomics, easily accessible serum or urine data are recorded longitudinally, personalized lifetime trajectories of individual patients can be predicted (Figure 2b).

SYSTEMS MEDICINE

A general aim of our lab is to translate preclinical findings into clinical routines as much as possible for the benefit of patients. Our NMR spectrometer is configured as a standardized IVDr (*in vitro* diagnostics research) platform, and we are collaborating with several clinical partners in many different fields. Hereby, we collected, prepared and analyzed hundreds of samples (blood, urine, liquor, feces or tissue) from large clinical trials based on their NMR signature for metabolites, lipoproteins and selected glycoproteins. We placed these results in context with our preclinical projects.

For instance, within a human glioma study, we used *ex vivo* NMR to obtain a deeper understanding of tumor metabolism and discovered several metabolites that cannot be detected by *in vivo* MRI or MRS (Figure 3a/b) but can be detected by *ex vivo* NMR (Figure 3c). Other major clinical collaborations include research related to Parkinson's and Alzheimer's disease, cardiac infarction, breast cancer, hepatocellular carcinoma, tumor senescence, metachromatic leukodystrophy, sepsis, rare diseases and more. Within the framework of an industrial collaboration with Bruker BioSpin GmbH, we are

working hand-in-hand to improve and validate diagnostic tests for clinical samples and are guiding our metabolomics lab toward integration into the international Phenome Centre Network. Recently, the analysis of immune cell extracts (Figure 4) and serum and urine samples from acute and long COVID-19 patients has become a major topic of our research, and thus, we are sharing spectra and expertise with additional NMR partners around the globe.

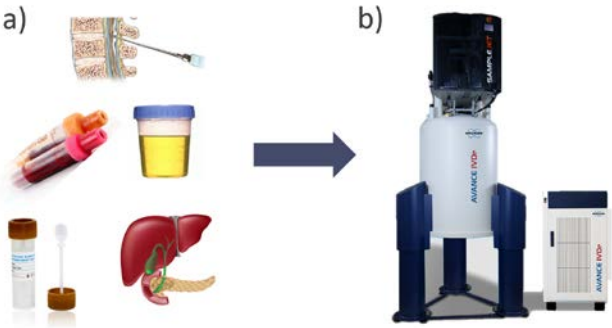


Figure 1: In metabolomics, the analysis of biological samples, such as liquor, blood, urine, feces and tissue, (a) with analytical instruments, such as high-field NMR spectroscopy, (b) quantifies small molecules of cellular pathways and places them in the context of health or disease.

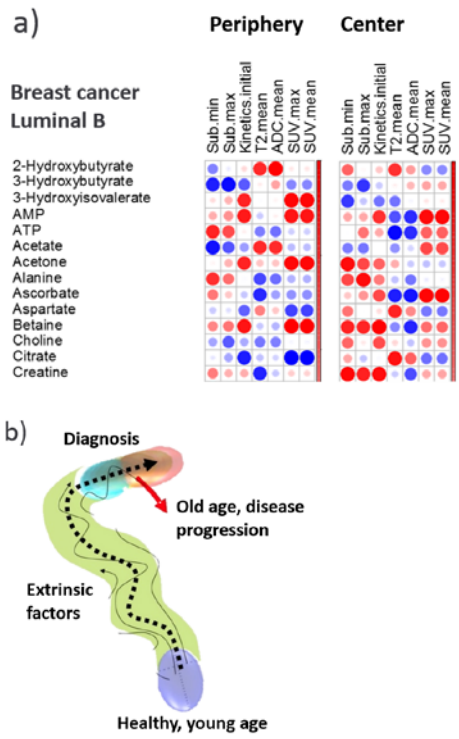


Figure 2: Correlation of breast cancer tissue metabolites from luminal B classified patients with imaging parameters (a) and schematic metabolic life trajectories from longitudinal sampled clinical cohorts (b). Abbreviations: ADC = Apparent Diffusion Coefficient, SUV = Standardized Uptake Value.

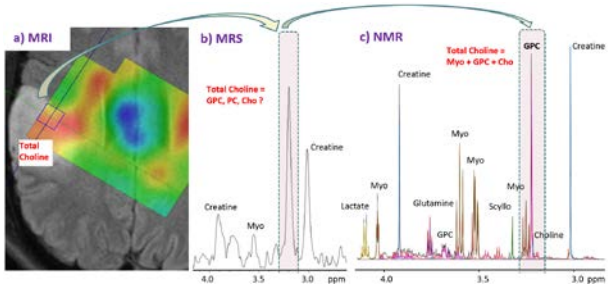


Figure 3: Magnetic resonance imaging (MRI) of a human glioma patient and signal maps of total choline distribution (a). A selected magnetic resonance spectrum (MRS) shows a high total choline peak (b), which by *ex vivo* nuclear magnetic resonance (NMR) spectroscopy could be deconvoluted into several individual metabolites (c). Abbreviations: GPC = glycerophosphocholine, PC = phosphocholine, Cho = choline, Myo = myo-inositol.

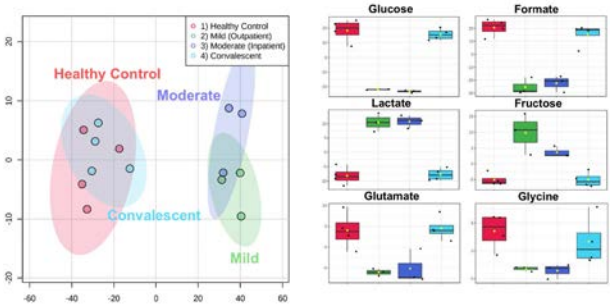


Figure 4: Principal component analysis (PCA) of immune cell extracts from healthy controls and COVID-19 patients. Altered energy metabolites between healthy controls and convalescent patients indicate a strong demand for glucose and a switch toward fructose utilization during the acute phase of viral infection.

Dr. Christoph Trautwein
Group Leader Metabolomics & Systems Medicine
+49-7071-29-83426
christoph.trautwein@med.uni-tuebingen.de

INFECTION & INFLAMMATION

Inflammation is a protective mechanism involving immune cells and occurs in response to tissue damage. While a normal acute inflammatory process addresses the cause of the damage, such as infection or trauma, and resolves itself by repairing tissue, it may become chronic and not resolve, resulting in loss of tissue function. This may happen during prolonged infections or autoimmune diseases and, while sometimes difficult, it is crucial to distinguish between sterile and nonsterile (e.g., bacteria, viruses) inflammation to provide proper care.

To efficiently characterize, address and monitor infection- and inflammation-related illnesses, there is a strong need for accurate imaging techniques that provide global insight into the state of the tissue and its environment; to this end, full-body imaging techniques such as PET, MRI and CT can be excellent modalities in both preclinical and clinical settings.

Our research aims to develop specific imaging tools for diagnostic and therapeutic monitoring of both sterile and nonsterile immune responses and inflammatory events through the development of specific radiotracers and methodologies at the preclinical level and their translation to the clinical setting. In particular, we are currently pursuing imaging research of several infectious and sterile lung diseases and of the potential roles of macrophages in cancer diagnosis and therapy.

FUNGAL INFECTION: INVASIVE ASPERGILLOSIS

Aspergillus fumigatus is a ubiquitous airborne mold whose spores are frequently inhaled. While a healthy immune system destroys the fungus, patients with a heavily impaired immune system (e.g., graft recipients, cancer patients under therapy, AIDS patients) are at risk of developing a severe fungal infection called aspergillosis, which results in rapid death in 50% of patients. Standard diagnosis is typically obtained using invasive approaches, such as biopsies, at a late disease stage. Within the EU-funded MATHIAS consortium, we developed the first specific and noninvasive diagnostic approach for invasive aspergillosis that uses antibody-based PET imaging (Figure 1); this approach is now being translated to the clinic. In addition, we are conducting preclinical studies to evaluate the potential of this image-guided approach for therapy monitoring.

LUNG FIBROSIS

Idiopathic pulmonary fibrosis (IPF) is a chronic disease that is invariably fatal, and patients are plagued by a life expectancy of less than five years. Characterized by a loss of function of lungs and bronchia due to general build-up of connective tissue, IPF is notably difficult to diagnose early, as its symptoms are shared with common benign respiratory tract diseases. While anatomic imaging of IPF is already a useful tool for confirming diagnosis, no molecular imaging tool is currently available to enable early differential diagnosis and allow for efficient treatment of the disease before tissue impairment starts. Building on the senescence imaging experience of the Werner Siemens Imaging Center, we are currently investigating the interplay between senescence and fibrosis during disease progression using PET, CT and MRI. In the second part of the study, we investigate the efficiency of senolytics as a potential IPF treatment option using newly developed imaging tools. We expect this study to identify potential candidates for the first clinical investigation of early IPF diagnostics using molecular imaging tools, ultimately providing a better outlook for patients.

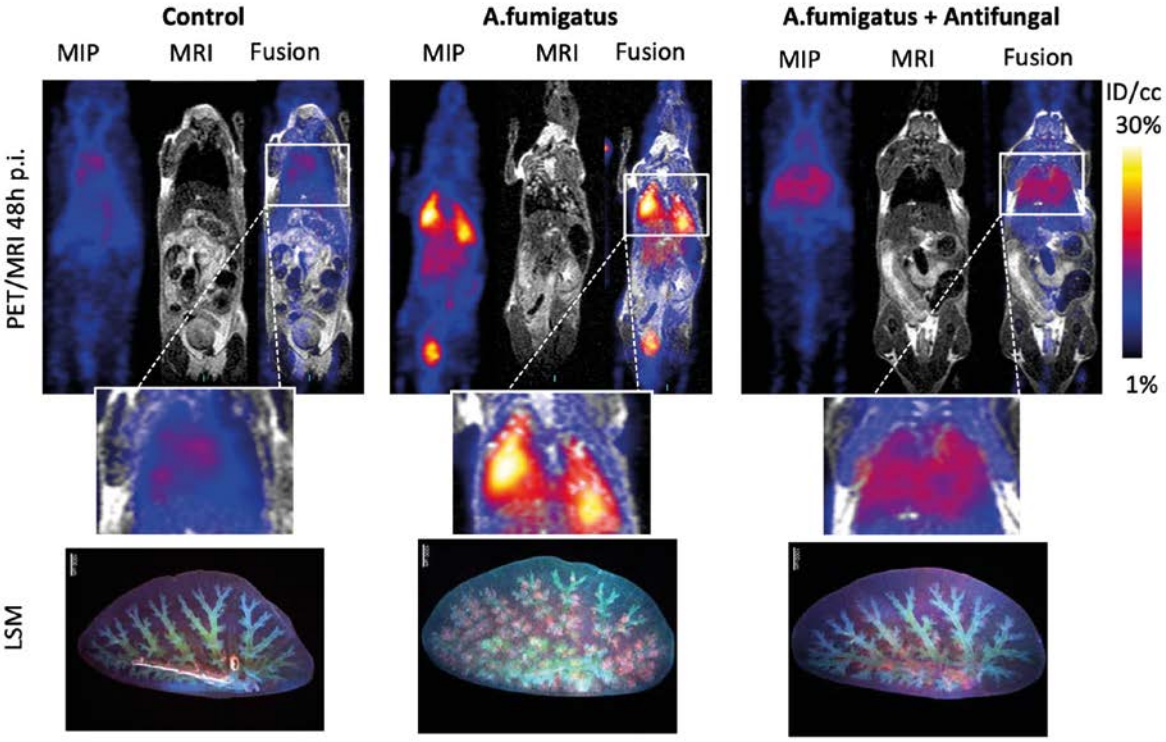


Figure 1: Invasive aspergillosis imaging. Top row: Sagittal maximum intensity projection (MIP) PET images, MR and fused PET/MR images of PBS-treated, A. *fumigatus*-infected and antifungal-treated A. *fumigatus*-infected mice injected with [^{64}Cu]Cu-NODAGA-hJF5-DyLight. Middle row: magnified PET/MR image of the thoracic cage. Bottom row: Light sheet microscopy (LSM) of the lungs infected with A. *fumigatus* (red) and [^{64}Cu]Cu-NODAGA-hJF5- DyLight (green) in the lung tissue (white). Tracer injection demonstrates high uptake in infected lungs compared to control lungs, while animals undergoing effective antifungal treatment show less accumulation of the radiotracer than untreated animals. (Image courtesy: cooperation partner Prof. Dr. Matthias Gunzer and Sophie Henneberg)

INVOLVEMENT OF THE IMMUNE SYSTEM DURING CANCER PROGRESSION AND THERAPY

Supported by the Excellence Strategy at the University of Tübingen and driven by a common funding program with the Molecular Imaging Program at Stanford, we are investigating the involvement of macrophages in cancer therapy and their potential use as a prediction marker and therapeutic target using novel radiotracers. As a first approach, we targeted the B7-H4 pathway, which is hypothesized to act as a backup checkpoint that prevents a proper immune response during cancer progression. Mapping of B7-H4 expression and its potential blockade is expected to reveal a new immune checkpoint inhibition strategy. As a second and parallel approach, we investigated how combining macrophage-targeted immunotherapy and radiotherapy can provide a synergistic antitumoral effect through the repolarization of macrophages. This requires tracking of the macrophage population in its entirety and of its specific subtypes and thus relies heavily on multimodal and multiparametric imaging approaches.

Dr. Nicolas Bézière
Group Leader Infection & Inflammation
+49-7071-29-87511
nicolas.beziere@med.uni-tuebingen.de

FUNCTIONAL AND METABOLIC BRAIN IMAGING

STUDY OF SYNAPTIC DYSFUNCTION IN ANIMAL MODELS OF NEUROLOGICAL DISORDERS USING SIMULTANEOUS PET/BOLD-FMRI

Structural disruptions and loss of synapses are major hallmarks of neurodegenerative disorders and result in network disruptions and loss of neuronal signaling. How synaptic dysfunctions appear early in the process of neurodegeneration is not yet understood. Our aim is to develop and apply protocols and methods (including pharmacological and optogenetic stimulations) to assess molecular changes in receptor expression by PET and functional changes by BOLD-fMRI at different disease time points in order to develop early readouts of disease progression (Figure 1). For this purpose, we used different rat models and genome engineering technologies (CRISPR/Cas9) to target specific genes and proteins *in vitro* (cell culture and primary neurons, Figure 2) and *in vivo* in the rat brain. For the analysis of PET and MRI data, we use several data analysis methods, including kinetic modeling and machine learning approaches.

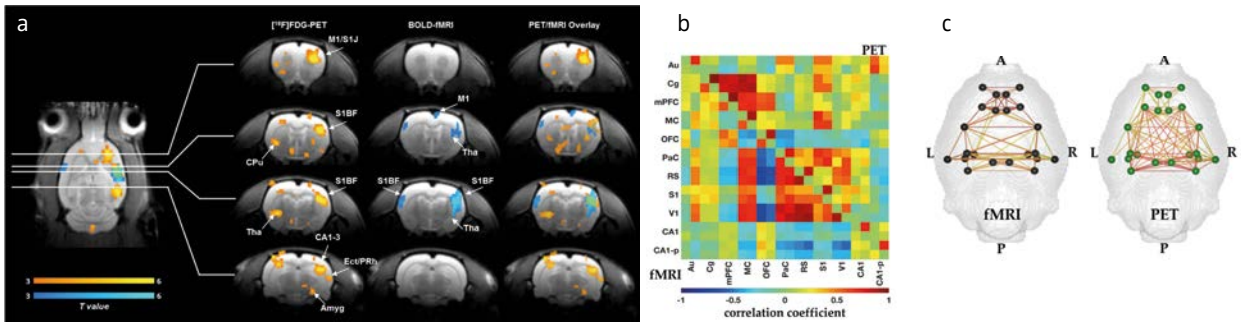


Figure 1: Simultaneously acquired [^{18}F]FDG PET and BOLD fMRI activation patterns after left whisker stimulation show functional and metabolic activity in S1 barrel field (S1BF) and thalamus (Tha) (a). Metabolic changes are observed in several additional brain regions (caudate putamen (CPu), hippocampal region (CA1-3), amygdala (Amyg) and ectorhinal cortex (Ect)). Correlation matrix (b) and functional and metabolic connectivities (c) of the default mode network in the resting state.

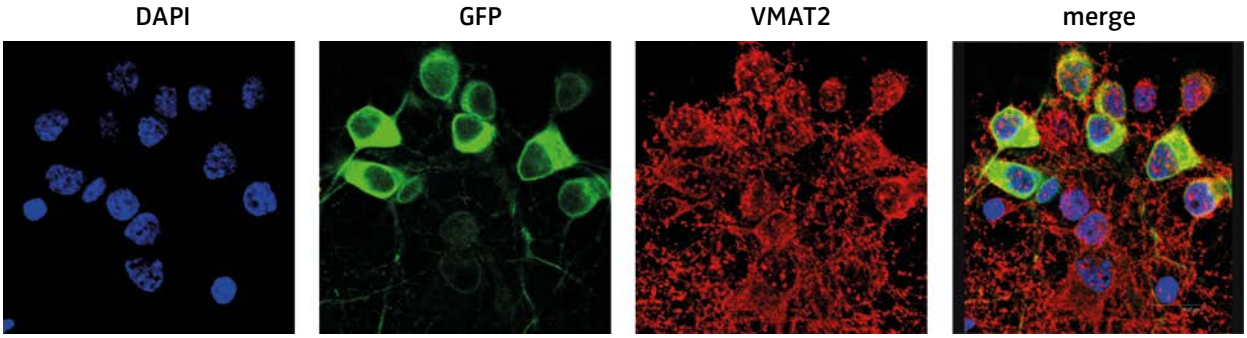


Figure 2: Immunofluorescent staining of rat primary neurons to study protein expression patterns after gene editing. DAPI staining shows cell nuclei, GFP reporter indicates plasmid expression in primary neurons and target expression (vesicular monoamine transporter 2 (VMAT2) as example).

TRACER DEVELOPMENT AND PRECLINICAL EVALUATION IN NEUROLOGY

In the past, we established several *in vitro* and *in vivo* screening assays to validate novel PET imaging agents (Figure 3). As our group maintains a strong collaboration with the radiopharmacy research group, we are always screening novel interesting targets in the brain. One important target is the protein alpha-synuclein, which plays a major role in the pathology of Parkinson's disease (PD). PET imaging of alpha-synuclein would be invaluable for noninvasive diagnosis of these diseases as well as facilitating the development of novel treatment strategies. However, in contrast to the situation in Alzheimer's disease, PET tracers to detect alpha-synuclein oligomers or aggregates in PD are still lacking. Therefore, we aimed to develop a PET tracer to noninvasively assess alpha-synuclein aggregation in the brains of patients with Parkinson's disease.

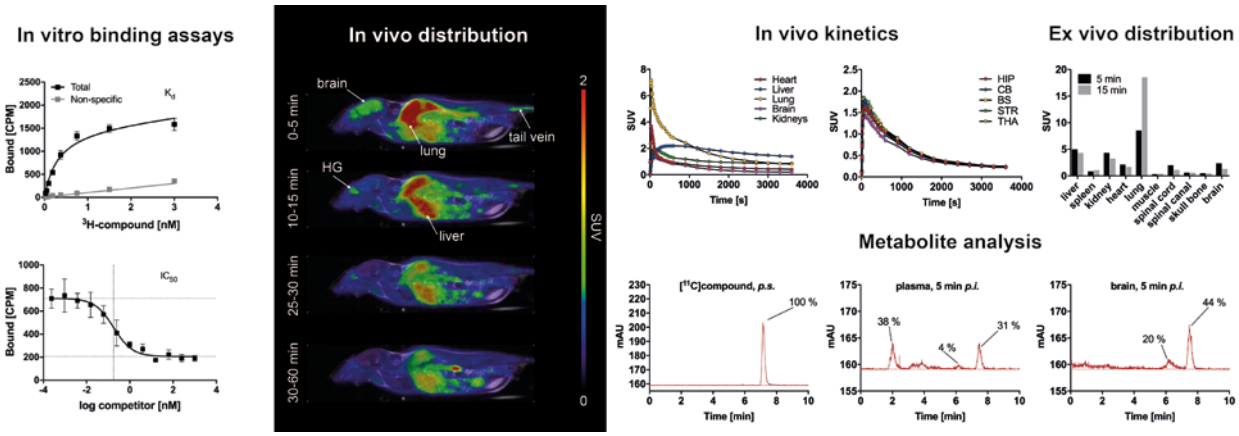


Figure 3: PET tracer development pipeline: compound screening using *in vitro* saturation and competition binding assays and recombinant fibrils for alpha-synuclein (αSYN), amyloid beta (A*β*) and Tau to determine target specificity and selectivity (left); *in vivo* PET imaging in mice to study tracer kinetics and blood brain barrier penetration (middle); and *ex vivo* biodistribution and metabolite analysis (right).

Prof. Dr. Kristina Herfert
Head of Functional and Metabolic Brain Imaging
(Receptor Imaging, Neurodegeneration)
+49-7071-29-87680
kristina.herfert@med.uni-tuebingen.de

IMAGING PROBE DEVELOPMENT

The development of new tracers and probes for PET and multimodal imaging is a vital step toward understanding unexplored disease-related biochemical pathways and advancing disease-specific diagnosis and research. The design, synthesis and optimization of such tracers and imaging probes as well as the development of synthetic processes for their production are critical steps in the establishment of new target-specific diagnostic imaging strategies.

In our state-of-the-art organic chemistry labs, new probes can be designed and synthesized from the ground up, allowing us to perform creative and innovative synthetic research in the fields of both small molecule and bioconjugate tracer development. New tracers and imaging probes are fully characterized and analyzed using a new Bruker 600 MHz NMR spectrometer featuring an autosampler and suitable probes for the relevant nuclei.

Our recently renovated radiochemical facilities, featuring functionally diverse synthesis modules in dedicated lead hot cells, allows us to radiolabel and produce new and routine tracers in-house for *in vivo* preclinical imaging research.

Close collaboration with imaging and clinical researchers, both internal and external to the Werner Siemens Imaging Center, ensures that our team remains at the pinnacle of cutting-edge radiotracer and radiochemical research.

Whether new probes for cellular senescence (a process vital to the inhibition of cancer growth), novel agents for the imaging of neurological disease, improved targeting of established biomarkers in diabetes or a host of other applications, our group focuses on building the molecular tools required for new and innovative imaging research.

PROJECT EXAMPLES

NEW PROBES FOR IMAGING CELLULAR SENESCENCE

Cellular senescence is broadly defined as the general biological program by which growth ceases, and it is accompanied by distinct changes in metabolic pathways. Senescence continues to gain recognition for its role in cancer treatment and therapy resistance. Treatment-associated senescence can be a measure of chemotherapeutic success, and the detection of senescent cells might also offer diagnostic opportunities for detecting precancerous lesions. In a project originally funded by the ERC project ImageLink that is now an integral part of the iFIT Cluster of Excellence, we are targeting biomarkers of senescence with newly developed radiotracers to quantify the contribution of senescence to successful cancer therapies.

IMAGING OF CELLULAR STRESS

Compared to healthy cells, cancer cells have to cope with a variety of additional challenges, which results in elevated stress levels. This cellular stress allows for the direct targeting of cancer with fewer side effects on healthy tissue. A prerequisite for the development and application of molecularly targeted therapy is the precise spatial and longitudinal assessment of stress states. Currently, we are focusing on evaluating tracers for biomarkers of replicative stress and on deregulated protein translation of cancer cells.

SYSTEMATIC ADAPTION OF ADVANCED RADIOCHEMICAL METHODOLOGIES

^{18}F is an attractive isotope for PET imaging due to its excellent imaging properties and practical half-life (110 min); however, the chemistry of the fluorine atom has traditionally been a limiting factor for the production of chemically diverse radiotracers. The advent of advanced transition metal-mediated radioflourination chemistry has begun to change this outlook by allowing radiochemists access to a powerful new radiochemical toolbox. These reactions are, however, multicomponent, complex and difficult to understand with respect to the large number of experimental factors critical to their success. We have thus started to explore these new tools through a statistical 'design of experiments' (DoE) approach, which is able to provide detailed information about these systems in an experimentally efficient manner. This has allowed us to learn more about the chemistry involved and how to best manipulate it so that we can quickly apply these new methods to accelerate the development and delivery of novel PET tracers to our imaging science projects.

Dr. Andreas Maurer
Group Leader Imaging Probe Development
+49-7071-29-87490
andreas.maurer@med.uni-tuebingen.de

RADIOPHARMACY

The use of radioactively labeled substrates began with Georg de Hevesy, who introduced the 'tracer principle' 90 years ago (for which he was awarded the Nobel Prize for Chemistry 1943). By introducing a radioactive label, a chemical compound (radiotracer) can be used to explore chemical or biochemical mechanisms by tracing the path of the underlying physiological processes.

Nuclear medicine is based on the tracer principle and is an important application of radioactivity in life sciences; in this field radiopharmaceuticals can be used for diagnostics and therapy. In nuclear medicine diagnostics, weakly radioactive and extremely small amounts of pharmaceuticals are applied. PET is an important method in modern molecular imaging. Molecules are labeled with positron-emitting radioactive atoms and are used to visualize biochemical and physiological processes in living organisms. PET diagnostics in oncology, immunology, cardiology or neurology are increasingly important tools on the path to personalized medicine.

Due to the very short half-lives of PET isotopes, radiopharmaceuticals for PET are regularly produced either on a daily basis or even for individual patient examinations. PET centers include a cyclotron (i.e., an accelerator to produce short-lived PET isotopes) and highly specialized laboratories to produce radiopharmaceuticals and are located in close proximity to tomograph(s) for PET diagnostics.

THE 6 STEPS OF RADIOPHARMACEUTICAL PRODUCTION

1. Radioactive isotopes for medical application are available either commercially (e.g., ^{131}I) or from a generator system that delivers the isotope on demand (e.g., ^{99m}Tc). In the case of PET, isotopes are produced by a cyclotron.
2. Radiolabeling, i.e., introduction of the radioactive isotope into a chemical substrate, is performed. After this radiolabeling, further reaction steps may be necessary, depending on the individual product.
3. The radiolabeled product is purified.
4. The product is formulated to achieve a solution normally for intravenous injection; in rare cases, oral administration may be possible.
5. Quality control of the radiopharmaceutical is performed.
6. The radiopharmaceutical is released for nuclear medicine application.

INFRASTRUCTURE AND EQUIPMENT

The PET center in Tübingen was established in 1995. The cyclotron (GE Healthcare, Sweden, 6 target positions, dual beam possible) can accelerate high energy protons (16.5 MeV) or deuterons (8.2 MeV) to produce ^{18}F (110 min half-life), ^{11}C (20 min), ^{13}N (10 min) and ^{15}O (2 min). In addition, the production of less commonly used isotopes, including ^{124}I (4 days), ^{86}Y (15 h) and especially ^{64}Cu (13 h), has also been established. Recently, the cyclotron has been upgraded to state-of-the-art technology (e. g., beam current 160 µA, high efficiency targets) to meet our requirements of maximum reliability and highest output. In addition, a generator is in place to produce ^{68}Ga (68 min) on demand.

To guarantee radiation safety and protect personnel and the environment during the production of radiopharmaceuticals, so-called hot cells have been installed; these hot cells are large boxes with at least 75 mm of lead shielding to minimize radiation doses in which the synthesis processes are performed. Inside the boxes, computer-controlled synthesizer modules are installed for automated production.

In further dedicated hot cells built as closed system isolators, products are sterile filtered under sterile conditions again to ensure the highest quality of the radiopharmaceuticals. Finally, in the isolator, samples are collected for quality control. Depending on the individual product, the final product batch may be divided into portions for various end-users (dispensing) by means of a robotic system inside the isolator.

Each product batch undergoes comprehensive quality control, ensuring that quality meets the specifications for the maximum safety of the radiopharmaceutical for the patient. Quality control includes testing for pH value, identity, radionuclide purity, chemical and radiochemical purity and microbial status, such as endotoxin content and sterility. The product is released for human administration by the Qualified Person (QP) in charge only when all the specifications are met.

OUR FACILITY

- Clean room laboratories (class C) for GMP production of radiopharmaceuticals with 10 synthesis hot cells containing the various automated synthesizers and 2 isolators (class A).
- Storage room for materials in a class D clean room.
- Laboratories for quality control (not classified), including 5 high-performance liquid chromatography systems, 2 gas chromatography systems with mass spectrometer and flame ionization detector, a phosphor imager, a high-performance gamma spectrometer, an endotoxin test device, a sterile filter integrity test, 2 pH meters and an osmometer.
- Laboratories with 4 hot cells for radiopharmaceutical development.
- Technical compartment with two compressing systems for radioactive gas waste storage.
- Central gas supply station (for the gases nitrogen, argon, helium and hydrogen). ➤

GOOD MANUFACTURING PRACTICE (GMP) PRODUCTION SITE

The production of radiopharmaceuticals for human application under a marketing license, manufacturing authorization or in a clinical trial (as with pharmaceuticals in general) must follow international GMP guidelines. The purpose of GMP is to confirm identity, strength and purity and to ensure the uniform quality and safety of a pharmaceutical product.

GMP, based on quality assurance of the system, encompasses everything that impacts the quality of the (radio-)pharmaceutical product (i.e., premises, personnel, equipment, raw materials, hygiene and monitoring, quality control and documentation).

Our recently completed GMP-based facility for radiopharmaceutical production meets the highest standards of the current GMP requirements in order to satisfy modern demands for products that meet the highest standards for availability, reliability and patient safety.

REGULATORY ASPECTS FOR HUMAN APPLICATION

In Germany, radiopharmaceuticals can be produced and applied in the context of four legal frameworks which fall under either the 'Medicinal Products Act' (Arzneimittelgesetz, AMG) or 'regulation on radioactive or ionizing radiation treated medicinal products' (AMRadV).

- Marketing license (AMG §21 ff).
- Clinical trial (AMG §40 ff).
- Clinical use of a compound known in the literature (AMRadV §2 Abs. 1).
- Production and use under direct responsibility of a physician (AMG §13 Abs. 2b).

For the first three regulations, a manufacturing authorization from the local authority is mandatory, and GMP rules must have to be followed. A marketing license is granted by the federal institution (BfArM) and allows for commercial distribution of the product.

OUR PRODUCTS AND WHAT THEY ARE USED FOR

Our radiopharmacy produces tracers and radiolabeled drugs not only for diagnostics (PET) but also for therapy, utilizing all four of the above mentioned possibilities. Products are used in-house for PET/CT and PET/MRI and are also offered to external customers outside Tübingen and to scientific collaboration partners. Under AMG §13 2b, external physicians may also produce a nonlicensed product for their patients in our laboratory with support from our staff.

AVAILABLE TRACERS

PET diagnostics

[^{18}F]FDG
Marketing license
Visualization of glucose metabolism, e.g., tumors
> 250 produced batches p.a.

[^{18}F]FPyGal
Manufacturing Authorization
Marker of senescence, tumors
ca. 30 batches p.a.

[^{18}F]FMISO
Manufacturing Authorization
Marker of hypoxia, tumors
< 10 batches p.a.

[^{18}F]PSMA-1007
Manufacturing Authorization
Marker of PSMA, prostate cancer
> 100 batches p.a.

[^{18}F]Fluoroethyltyrosine (FET)
under AMG §13 2b
Marker of amino acid transport, brain tumors
ca. 50 batches p.a.

[^{18}F]Fluoroethylcholine (FEC)
under AMG §13 2b
Marker of proliferation, prostate cancer, parathyroid gland
ca. 20 batches p.a.

[^{18}F]Fluoride
under AMG §13 2b
Marker of bone uptake, bone metastases
ca. 10 batches p.a.

[^{18}F]Fluorothymidine (FLT)
under AMG §13 2b
Marker of proliferation, tumors
< 10 batches p.a.

[^{11}C]Methylphenidate
Manufacturing Authorization
Marker of dopamine transporter, neurology, psychiatry
ca. 50 batches p.a.

[^{11}C]PIB
Manufacturing Authorization
Marker of beta-amyloid plaques, Alzheimers disease
ca. 30 batches p.a.

[^{11}C]Raclopride
Manufacturing Authorization
Marker of dopamine-D2-receptor, neurology, psychiatry
ca. 30 batches p.a.

[^{11}C]Methionine
Manufacturing Authorization
Marker of amino acid utilization, brain tumors
ca. 10 batches p.a.

[^{11}C]Choline
Manufacturing Authorization
Marker of proliferation, prostate cancer
< 10 batches p.a.

[^{64}Cu]Cu-NODAGA-hJF5-AK
under AMG §13 2b
Marker of aspergillus infection
< 10 batches p.a.

[^{64}Cu]Cu-NOTA-GD2-AK
under AMG §13 2b
Marker of GD2 antigen, neuroblastoma
< 10 batches p.a.

[^{64}Cu]Cu-NOTA-GPVI
under AMG §13 2b
Marker of atherosclerotic plaques, cardiology
< 10 batches p.a.

[^{64}Cu]Cu-DOTA-CD19-AK
under AMG §13 2b
Marker of CD19 antigen, tumors
< 10 batches p.a.

[^{89}Zr]Zr-Df-IAB22M2C
Manufacturing Authorization
Marker of CD8 antigen, tumors
ca. 50 batches p.a.
in clinical trials
first production site of a CD8 tracer in
Europe, production for EU and UK

Therapeutics

[^{177}Lu]Lu-PSMA I&T
under AMG §13 2b
Marker of PSMA, prostate cancer
> 100 batches p.a.

[^{177}Lu]Lu-HA-DOTATATE
under AMG §13 2b
Marker of somatostatin receptors, neuroendocrine tumors
> 50 batches p.a.

[^{90}Y]Y-HA-DOTATATE
under AMG §13 2b
Marker of somatostatin receptors, neuroendocrine tumors
< 10 batches p.a.

One main focus of our department is to bring newly developed PET radiopharmaceuticals to the patient. This translational research is intended to further provide clinics with highly specific and selective biomarkers, enabling the visualization of new relevant target structures and mechanisms, thereby reinforcing the applicability of PET diagnostics for the benefit of patients.

Apl. Prof. Dr. Gerald Reischl
Head of Radiopharmacy
+49-7071-29-80531
gerald.reischl@uni-tuebingen.de

CLINICAL TRANSLATION

A major goal of our group is to facilitate the use of our basic research results to improve clinical health care. Novel radiotracers, imaging probes, imaging technologies, experimental techniques and approaches follow a long methodological and rigorous path from the preclinical setting to the clinic. Therefore, it takes a multidisciplinary team of biologists, chemists, physicists, clinical scientists and clinicians, including physicians, technicians, study nurses and many others, who work hand-in-hand to successfully develop, evaluate and apply novel approaches in the clinical setting.

Our facility has developed strong relationships with several clinical partners in recent years, such as the Department of Nuclear Medicine and Clinical Molecular Imaging, Department of Diagnostic and Interventional Radiology, Department of Neuroradiology, Department of Medical Oncology and Pneumology, and the Department of Dermatology.

The possibility of joint training for physicians in the dedicated MD/PhD 'Experimental Medicine' program of the Faculty of Medicine in both basic research and clinical residency ('Facharztweiterbildung') helps to bridge the gap between the preclinical experimental setting and the daily clinical routine. Currently, three physicians are actively working as postdocs and group leaders of their own research team in the Werner Siemens Imaging Center as well as residents in different clinical departments.

ADVANCED IMAGE ANALYSIS

An important field for translational research is the implementation of advanced image analysis tools in routine clinical practice. A Gaussian mixture model was able to predict histological subtypes in preclinical studies using mouse models of colon and breast cancer, which could be confirmed in a clinical pilot study. These promising results led to the initiation of a prospective clinical trial, which is currently running in close collaboration with the Department of Gynecology, Department of Nuclear Medicine and Clinical Molecular Imaging and the Department of Diagnostic and Interventional Radiology. Along this line, more sophisticated algorithms, including advanced machine learning methods, are currently implemented in imaging workflows and validated, including preclinical and clinical datasets (Figure 1).

NOVEL IMAGING TRACERS

The development of new tracers is a prerequisite to obtain deeper insights into the molecular mechanisms of diseases, but it is also the next step in improving diagnostic accuracy and treatment stratification. In recent years, we developed various tracers in the Werner Siemens Imaging Center, which are now, after extensive preclinical testing, being applied in the clinic under the framework of compassionate use or prospective clinical trials. The newly developed senescence tracer is able to detect senescent tissue, e.g., in tumors. Senescent tumor cells are in a state of growth arrest but may accelerate tumor growth by secreting proliferative mediators. Targeted treatment with senolytic drugs is able to specifically target these cells (Figure 2, left).
Radiolabeled antibodies, such as anti-GD2 or anti-CD19, enable the specific detection of antigen expression in tumors and metastasis, which is essential for guiding innovative immunotherapy approaches, such as CAR T cells (Figure 2, right.). Such radiolabeled antibodies might also be used for future theranostic approaches that link diagnosis and therapy by using the same biological antibody radiolabeled with different isotopes.

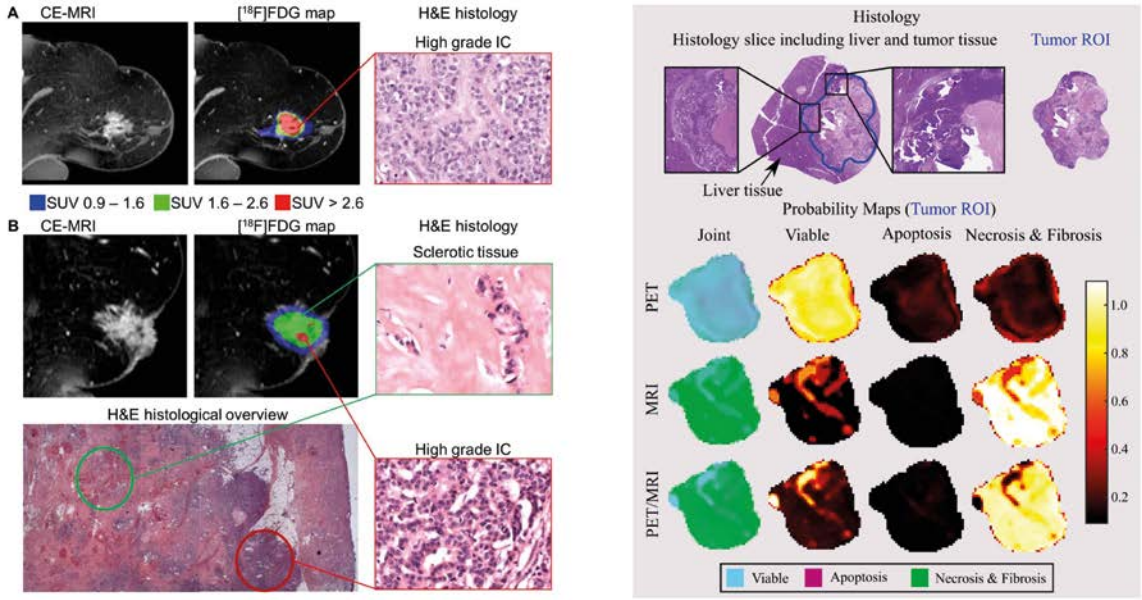


Figure 1: (left) Breast tumors imaged by combined PET/MRI and analyzed by a Gaussian mixture model to reveal morphological intratumoral variations, confirmed by *ex vivo* histology. Schmitz et al., Cancer Res. 2016;76(18); (right) Translational study in colorectal cancer patients applying PET/CT imaging and machine learning algorithm developed on preclinical mouse models. Katiyar et al., manuscript in revision

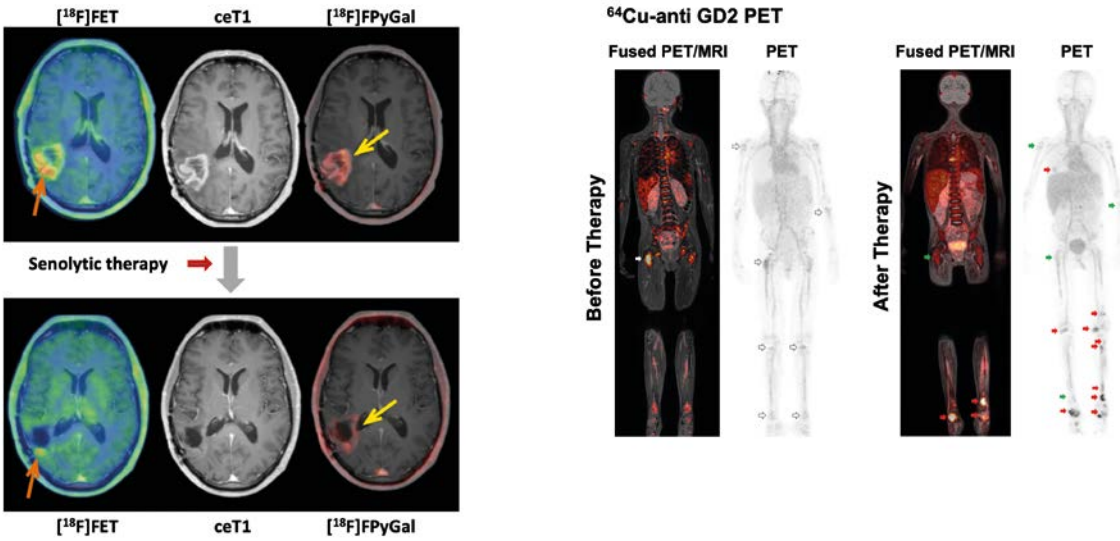
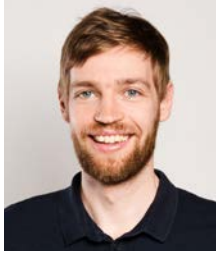


Figure 2: (left) Senescence imaging of glioblastoma using a novel [18F]FPyGal-Tracer. Cotton et al., manuscript in preparation; (right) GD2 immune imaging of a young patient with neuroblastoma; arrows indicate tumor lesion. Schmitt et al., manuscript in preparation

Dr. Salvadore Castaneda Vega
Group Leader Translational Neuroimaging
+49-7071-29-87479
salvador.castaneda@med.uni-tuebingen.de



Dr. Johannes Schwenck, MD, PhD
Group Leader Translational and Preclinical Imaging in Inflammation & Cancer
+49-7071-29-86870
johannes.schwenck@med.uni-tuebingen.de

Dr. Dominik Sonanini
Group Leader Translational Immunoimaging & Cancer Immunotherapies
+49-7071-29-87589
dominik.sonanini@med.uni-tuebingen.de

IMAGING SCIENCE TECHNOLOGIES

The Werner Siemens Imaging Center offers 245 m^2 of restricted imaging and animal holding laboratories with elevated hygiene. The entire sector is equipped with the latest air conditioning technology and high-efficiency particulate air filters. Personnel enter the restricted area via a clean room through an air shower. The laboratory has been approved for biosafety level 2 (S2) work and as a radiation area, enabling the use of all commonly used radioactive isotopes for PET and SPECT imaging.

The latest state-of-the-art equipment for noninvasive functional *in vivo* imaging is located within this restricted area. Two 7 Tesla dedicated small animal MRI tomographs (BioSpec, Bruker BioSpin GmbH, Ettlingen, Germany) are accompanied by three dedicated small animal PET scanners (Inveon, Siemens Healthineers, Knoxville, USA), one small animal SPECT/CT scanner (Inveon, Siemens Healthineers), two optical imaging systems (Aequoria, Hamamatsu Photonics, Herrsching, Germany and IVIS Spectrum Imaging System, PerkinElmer, Waltham MA) and a DNP hyperpolarizer (SpinLab, GE Healthcare, Boston, USA).

The two MRI scanners are equipped with a multinuclei option to investigate adenosine triphosphate (ATP)-levels (^{31}P) and sodium-channel activity (^{23}Na), to follow ^{19}F-labeled cells, or to monitor pyruvate-lactate conversion using hyperpolarized ^{13}C magnetic resonance spectroscopy (MRS). One MRI is further equipped with a fully integrated PET insert that allows the simultaneous investigation of different metabolic processes using independent modalities. Furthermore, the combined information of PET and MRI may be used to investigate similar biomarkers (e.g., choline levels from MRS and [^{11}C]Choline PET) and elucidate in great detail the mechanism of the corresponding signal.

Three small animal PET scanners allow *in vivo* investigations in the millimeter range and absolute quantification of the acquired data. All systems are equipped with ^{57}Co sources to enable attenuation correction of the acquired emission data. In addition, the systems allow the monitoring of physiological parameters, such as heart and breathing rates. High-end animal bed solutions with constant and stable temperature regulation and monitoring, anesthesia supply and stereotactic holders ensure the reproducibility and reliability of the ac-

quired data among all our preclinical imaging systems. In addition to the PET scanners, a small animal SPECT/CT scanner enables the acquisition of tracers in the submillimeter range in combination with the high-resolution anatomical information gained by CT. A substantial advantage of these systems is that the PET scanner can be mounted to the SPECT/CT scanner to enable sequential PET/SPECT/CT studies. CT is capable of acquiring resolutions in the µm range, also enabling high-resolution *ex vivo* determination of bone structures in mice and rats.

Two optical imaging (OI) systems allow the acquisition of state-of-the-art research in the fields of oncology, cardiology, neurology, infectious diseases and inflammation due to their high sensitivity, signal-to-noise ratio, image analysis options and quantification possibilities.

This latest state-of-the-art technology platform offers the possibility of a wide range of *in vivo* imaging in combination with quantification of the acquired data.

In addition to the variety of different *in vivo* imaging modalities, the Werner Siemens Imaging Center is also well equipped with a 600 MHz nuclear magnetic resonance (NMR) system (Avance III, Bruker BioSpin GmbH) for *ex vivo* tissue or fluid analysis. The NMR is equipped with a solid-tissue probe (suited for magic angle spinning spectroscopy) to allow the investigation of various tissue samples. It is fully integrated into the laboratory, which also allows the analysis of radioactive material. In addition to its use for *ex vivo* verification of *in vivo* MR spectroscopy profiles, it can be used to elucidate therapeutic responses and provide deeper insight into tumor metabolism and viability.

In addition to the *in vivo* and *ex vivo* imaging modalities, the Werner Siemens Imaging Center is equipped with all standard *in vitro* and *ex vivo* analysis tools, such as

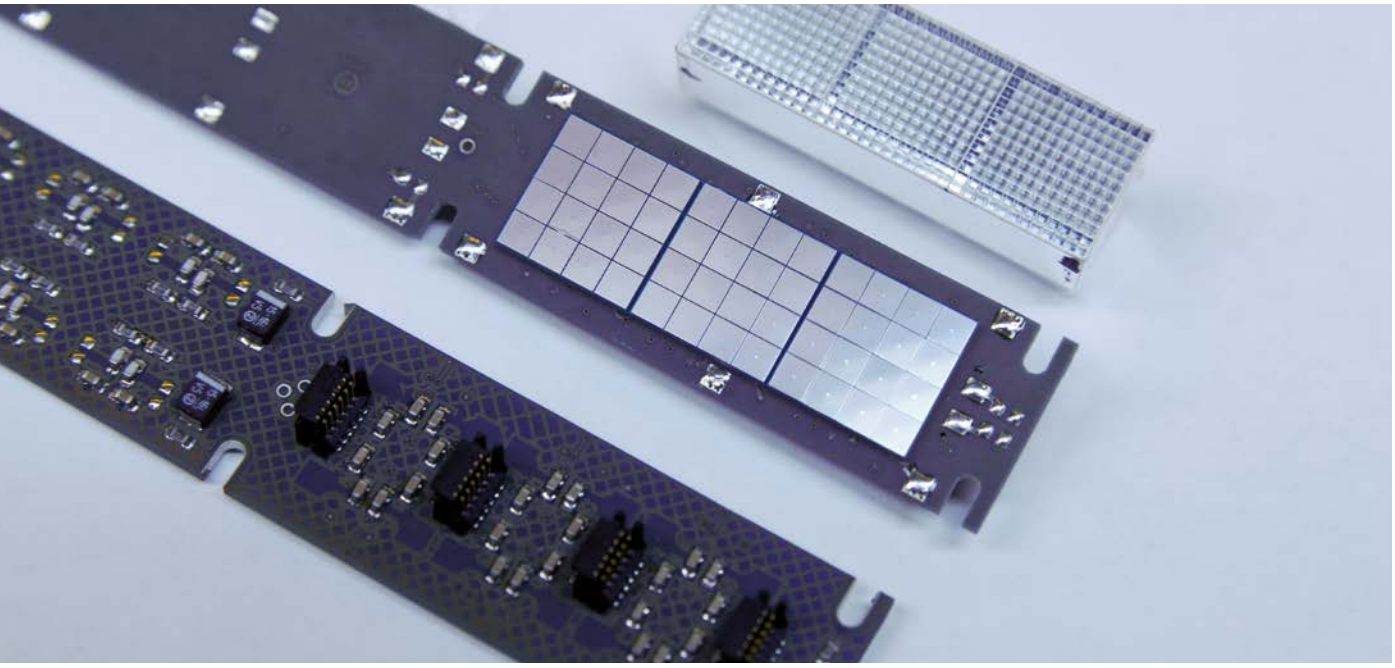
- blood gas analysis,
- ELISA,
- RT–PCR,
- BLOT technology,
- autoradiography,
- gamma counting,
- immunohistochemistry,
- and many more...

The maintenance of three strictly separate cell culture labs for human, murine and transfected cells, along with regular established mycoplasma tests, eliminate the risk of bacterial cross-contamination between cell lines.

The Werner Siemens Imaging Center has demanded that it meets the highest laboratory standards. Routine quality control checks are implemented and constantly verified, and standardized protocols for both *in vivo* and *ex vivo* techniques are in place, to ensure the highest accuracy, reproducibility and reliability of the acquired data.

Dr. Andreas Schmid
Group Leader MR & Multimodal Imaging Science
+49-7071-29-87510
a.schmid@med.uni-tuebingen.de

Dr. Julia Mannheim
Group Leader PET & Multimodal Imaging Science
+49-7071-29-82974
julia.mannheim@med.uni-tuebingen.de



IMAGING DETECTOR PHYSICS

To be able to determine the answers to the increasingly complex questions in the field of preclinical and clinical imaging, a continuous advancement of the underlying technologies is required. For this reason, our group is working on innovative hardware and software developments that support the systems used for molecular imaging. Our key competence is the development of novel PET detectors that are used for clinical and preclinical systems.

COMBINED PET/MR FOR BREAST IMAGING

One example is the development of a PET insert that can be used for breast imaging within a clinical 3T PET/MR. The detectors were specifically designed by our group to withstand the harsh conditions inside an MR scanner, i.e., the strong magnetic field and transmission of powerful electromagnetic waves. Furthermore, the electronics of the PET detectors transmit electromagnetic waves, which may lead to a distortion of the sensitive receiver technology of the MR scanner. To ensure mutual compatibility between the PET insert and the MR scanner, continuous tests of several hardware iterations inside the clinical MR scanner were required, as shown in Figure 1. To achieve a high performance of this PET system, our group is using state-of-the-art technologies, such as silicon photomultipliers (SiPMs), application-specific integrated circuits (ASICs), and field programmable gate arrays (FPGAs). To identify the optimum design for the breast PET/MR insert, we began comprehensive simulations of different geometries for the PET system at an early stage of building the system, as shown in Figure 2. The evaluation of the performance parameters of this novel PET system requires adapted image reconstruction and an extension of the existing characterization standards. As such, our group utilizes the GATE framework based on GEANT IV, which is a powerful tool for Monte Carlo simulations of high-energy particles in the field of PET. New insights gathered from the simulations are directly transferred to the hardware development and vice versa, i.e., limitations of the PET hardware are included in the simulations to create a model that is as close as possible to the final PET system.

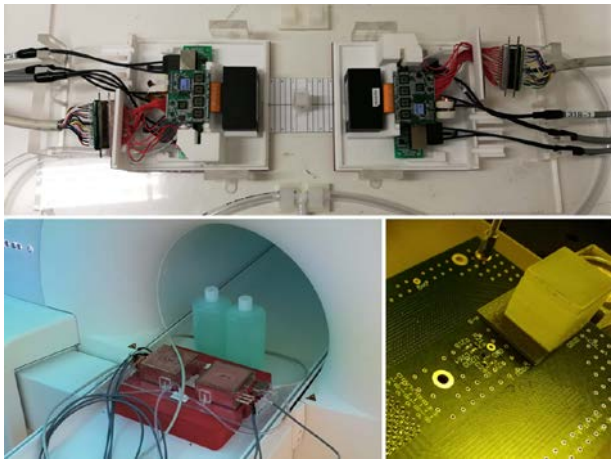


Figure 1: On the top: Two opposing prototype PET detectors with a centered Na- 22 radiation point source. This setup enables the measurement of coincident events, as would be the case for the detectors of the ring of a PET system and originating from the radioactive decay of the isotope of the PET tracer inside a patient; on the bottom left: Setup for the mutual compatibility test of PET detectors inside a clinical 3T MR scanner. The PET detectors are encapsulated in shielding boxes and utilize data communication via optical fibers. On the one hand, these methods ensure robustness against electromagnetic waves transmitted by the MR scanner. On the other hand, they help to prevent a distortion of the sensitive MR receiver coil by the radio frequency distortion emitted by the PET detectors. On the bottom right: Prototype PET detector composed of a scintillation crystal block placed on a photosensor array for the detection of incident gamma rays.

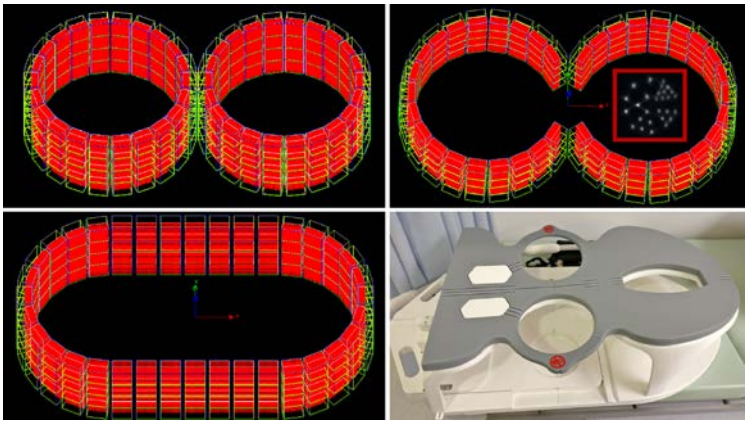


Figure 2: 3D rendered GATE models for three different geometries of the breast PET/MR insert. The individual scintillation crystal blocks of a single PET detector are visualized in red. A reconstructed image based on simulations of a Derenzo phantom is shown in the red box. On the bottom right: The structure of the PET system is designed to fit into the breast coil, which is used for imaging of the breast with an MR scanner.

ADVANCING PRECLINICAL IMAGING TECHNOLOGY

Other examples of the innovative work of our group are developments designed to overcome a crucial limitation of current preclinical scanners, namely, restraining the rodent during a scan. We support the potential outcome of behavioral and neurological studies by enabling imaging of unrestrained rodents, i.e., rodents that freely move in their cages. Therefore, our group is working on accurate motion correction of acquired PET data. To accurately measure the distribution of the PET tracer, our group first uses an optical tracking system to locate the exact position of the rodent. In a second step, machine learning methods are used to generate a 3D reconstructed model of the rodent from the multiview 2D images acquired over the scan time. The 3D reconstructed models are based on a dataset of scans from a variety of specimens, positions and imaging modalities, as shown in Figure 3. The anatomical information derived from the scans is used to enhance the 3D models to emulate the motion and deformation of a rodent.

In addition to improving PET imaging based on conventional technology, our group is constantly eager to think out of the box. This involves tests of new materials for suitability in the field of PET imaging as well as novel approaches, e.g., the detection of Cherenkov photons to improve the intrinsic spatial resolution of PET.

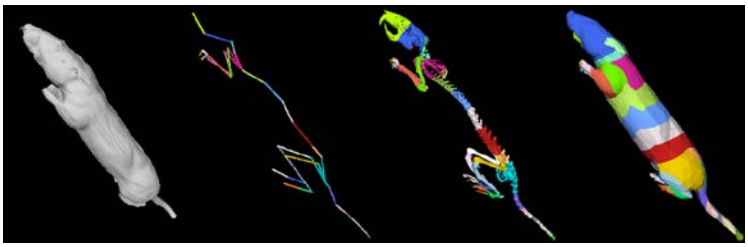


Figure 3: Example of the segmentation of the skeleton and tissue of the 3D rat model by a defined rig, which is determined from joints and bone sections to emulate the movement and pose-dependent deformations of the rat.

Dr. Fabian Schmidt
Group Leader Imaging Detector Physics
+49-7071-29-87481
f.schmidt@med.uni-tuebingen.de

MULTIPARAMETRIC DATA ANALYSIS & MINING

Modern imaging devices can generate large amounts of data, especially in a multimodality setting. For example, multiple functional MRI parameters can be acquired along with the dynamic and static uptake of PET tracers. The analysis of such datasets can be overwhelming for researchers and clinicians, and machine learning methods are used to increase the amount of useful information that can be extracted. We aim to find patterns in imaging data that allow us to differentiate between different tumor classes (e.g., benign and malignant), detect neurological abnormalities (e.g., stroke) or to differentiate between disease outcomes.

In oncological imaging, we specifically focus on heterogeneity, an important characteristic of tumors. Tumor heterogeneity represents the phenotypic variations that commonly exist between different regions within a tumor. The differences can influence the effectiveness of therapy or be a predictor for disease progression. Therefore, we are interested not only in the tumor as a whole but also in tumor heterogeneity. Imaging can help to elucidate the biological factors underlying these variations, as they can be observed with PET and MRI. However, most imaging methods do not provide all the necessary information directly, as a multitude of factors affect the image. Interesting information can be obtained by combining different imaging techniques and analyzing them simultaneously using machine learning methods.

To actually learn anything, imaging data are required, and some of the data need to be labeled. For example, a human expert can indicate which parts of the image meet certain criteria, or this information can come from another data source. In our case, we rely heavily on histology as the gold-standard with the expert knowledge of a pathologist. With this, we can train an algorithm to detect certain patterns in the images and are then able to apply the model to new data. It is even possible to perform training on data obtained in animals and predict imaging data from patients. Figure 1 shows an example of this translation to the clinic.

Slices or biopsies from a tumor can be analyzed with different histological stains, each providing different information about the tissue. We seek to apply the same methods on histological images as well: using machine learning to evaluate tumor heterogeneity.

The different datasets acquired in the clinic as well as in the Werner Siemens Imaging Center include multiparametric imaging data, histology data and data from various omics techniques. The comprehensively analysis of these data of different formats and origins adds another level of complexity. The data are processed and analyzed with a variety of computer algorithms and stored for later access. We are building a complete workflow for processing, storage, analysis and access. Integrating different sources of data will leverage their complementary information.

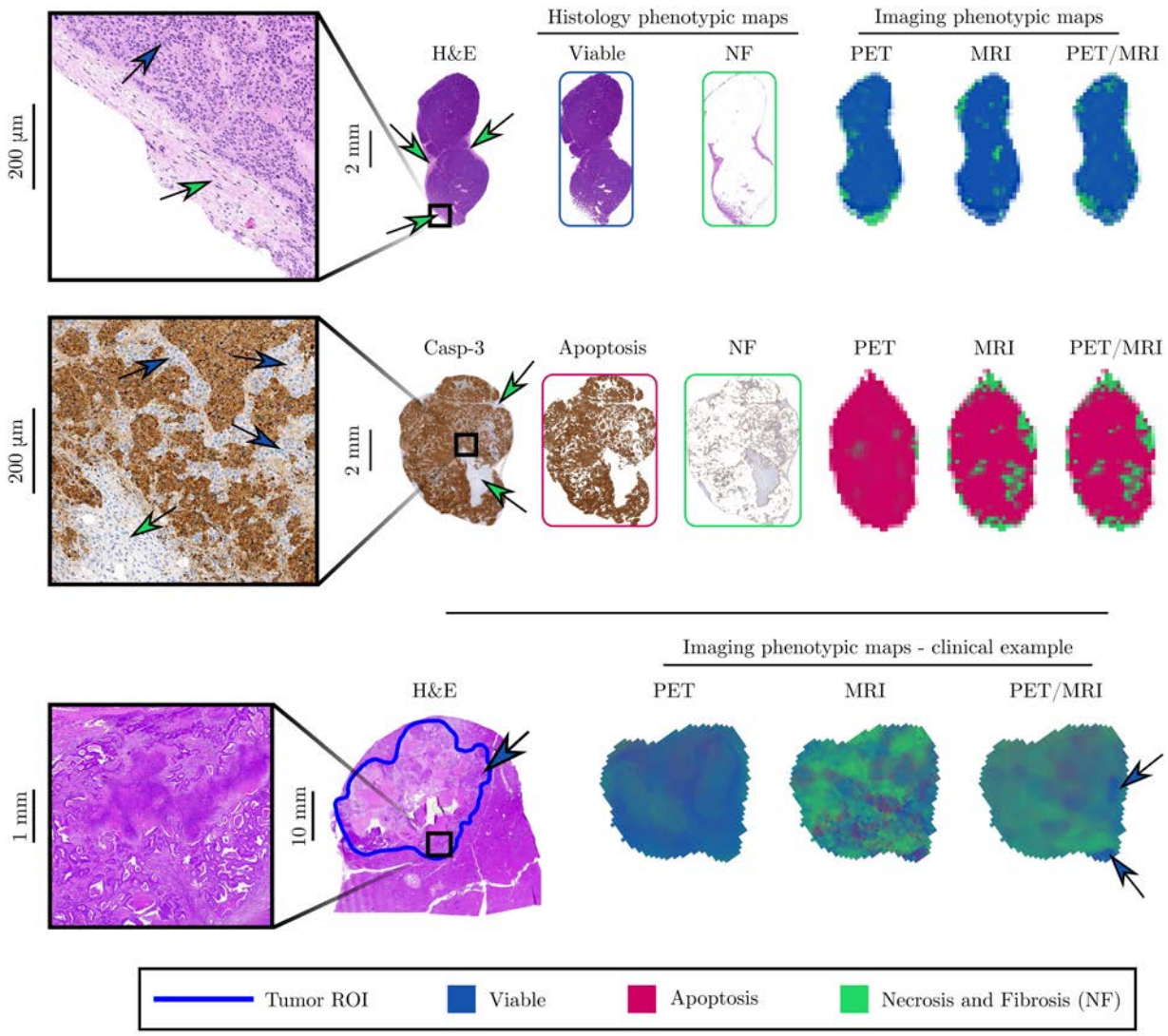
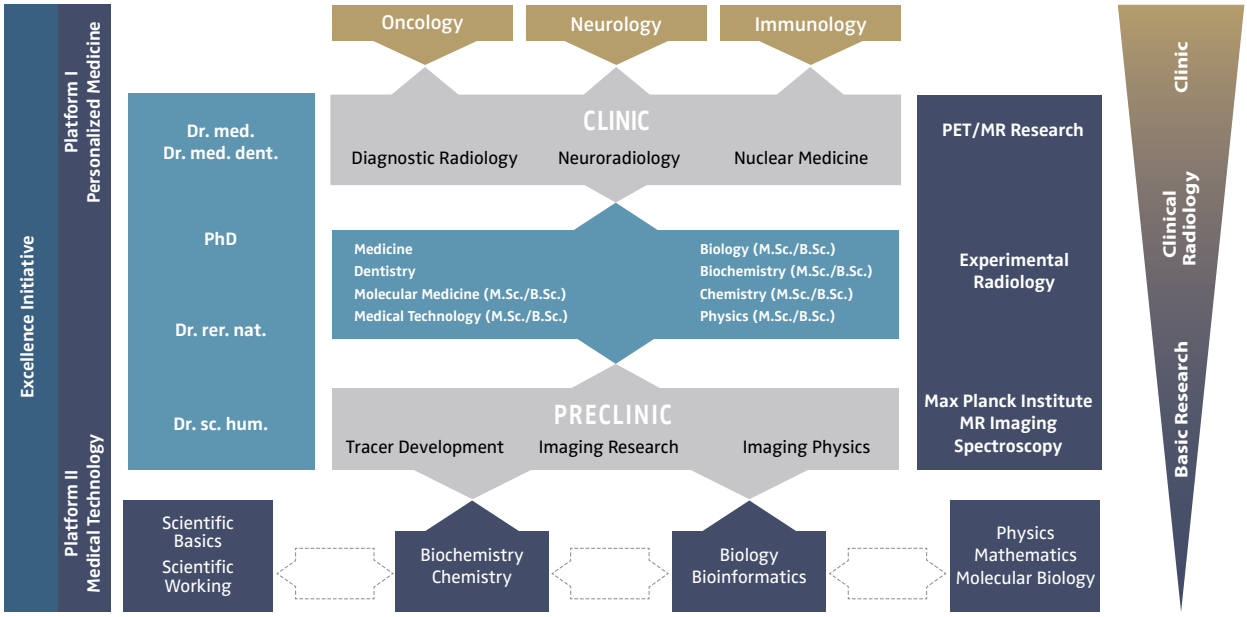


Figure 1: The top panel in the figure shows imaging and histology data of nontreated (top row) and treated (center row) mice with subcutaneous colon cancer. The histology phenotypic maps were used as a reference to segment PET/MRI data of the two tumors and obtain imaging phenotypic maps. The imaging phenotypic maps of many such tumors were used to train a machine learning model that could be applied to clinical PET/MRI data. The bottom panel shows clinical imaging phenotypic maps of colon cancer liver metastasis, and these maps were derived using the classifier trained on the preclinical mouse data. Here, the model trained on the PET/MRI data is able to accurately identify regions of viable tumor tissue (indicated with blue arrows) in an otherwise necrotic tumor.

Prof. Dr. Bernd Pichler
Group Leader Multiparametric Data Analysis & Mining
+49-7071-29-83427
bernd.pichler@med.uni-tuebingen.de

ACADEMIC TEACHING

As one of the leading worldwide facilities in preclinical imaging, the Werner Siemens Imaging Center is fully aware of its responsibility to provide students and young investigators with the opportunity to acquire expertise in the field of imaging science.



COLLEGE EDUCATION – CURRICULA

In the realm of imaging science, we have established various curricular teaching courses within Medicine, Medical Technology, Molecular Medicine and a PhD class on Experimental Medicine.

MEDICINE

Imaging modalities, including PET, CT, MRI and optical imaging (OI), as well as combined multimodal PET/MR or PET/CT technologies offer great benefit not only in biomedical research but also to all clinical fields in modern medicine. Therefore, in this module, we focus on teaching the basic principles of imaging science, including tracer production and the underlying radiochemistry, to enable upcoming physicians to gain a broader understanding of the clinically available imaging modalities and to stimulate their interest in preclinical and translational imaging science.
Teaching language: German

BIOMEDICAL TECHNOLOGIES (BACHELOR'S & MASTER'S COURSES)

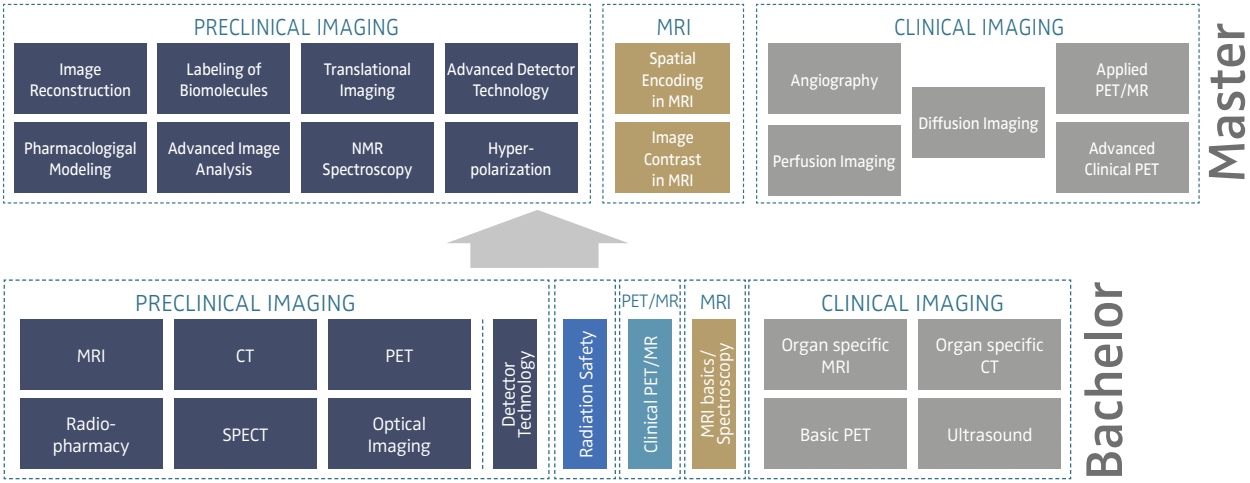
Education in the field of imaging science is based on a multidisciplinary and strongly interactive structure combining physics, biology, chemistry and medicine, bridging preclinical and translational research, as well as clinical science and routine diagnosis. Within this area, different imaging modalities have recently been developed, such as state-of-the-art combined PET/MR devices or cutting-edge OI systems. Further knowledge about the application of these innovative new technologies is of great benefit for all students of biomedical sciences. To meet these challenges, our group is strongly anchored within a network that includes various departments at the University and the University Hospital Tübingen and external academic and industrial partners. This interaction has led to one of the first interuniversity programs in the life sciences in Germany in cooperation with the competence areas of Medical Engineering (University of Stuttgart) and Biomedical Technologies (Tübingen, www.uni-medtech.de). Within this framework, the Department of Preclinical Imaging and Radiopharmacy is heavily involved in the teaching, the preparation and the execution of various practical courses.

BACHELOR'S COURSE

This module incorporates all of the various outstanding imaging modalities used in modern radiology, nuclear medicine and preclinical imaging science with a focus on detector technology, radiation safety and high-resolution noninvasive imaging modalities, such as MRI, PET, CT, SPECT and OI. Functional imaging modalities, such as PET, MRI or OI, are especially suited to visualize important physiological processes *in vivo* and are therefore important tools in the evolving field of personalized medicine. Here, students receive an overview of almost all modern and state-of–the-art imaging technologies and have the opportunity to experience an intense hands-on training course on the different imaging modalities used in routine clinical and preclinical research. A large portion of the practical classes focus on imaging technology, including particle detector physics and analog electronics for imaging applications. Digital signal processing, data handling and imaging reconstruction complement this module.
Teaching language: German

MASTER'S COURSE

The goal of this advanced elective module in Biomedical Technologies is to build upon students' basic level of knowledge in preclinical imaging (bachelor's program). After learning the advanced principles of multimodal and functional imaging, the students acquire all of the skills necessary to develop their own novel experiments and appropriately analyze the acquired data. The idea behind this approach is to provide students with the strongest possible skills for their master's thesis as well as to give them deeper insight into the fascinating field of preclinical and clinical bioimaging, including pharmacokinetic modeling using quantitative PET data and advanced MR technologies such as hyperpolarization and NMR.
Teaching language: English



MEDICAL RADIATION SCIENCES (MASTER'S COURSE)

One of the most recent training programs offered by our department is part of the innovative course of Medical Radiation Sciences. This course imparts theoretical and practical skills in the fields of medical physics, radiation biology and tumor biology as well as noninvasive preclinical imaging. Renowned scientific and clinical researchers focused on basic research teach the material, enabling all students to gain deeper insight into the field of medical radiation sciences. Furthermore, graduates have the opportunity to continue their education to qualify as an expert in medical physics. These additional two years allow students to become accredited and certificated specialists in all radiological-related fields of modern medicine, such as nuclear medicine, radiation therapy, radiology and radiation physics. A specialist of this kind is mandatory within all clinical institutions due to current legal regulations.

Teaching language: German/English

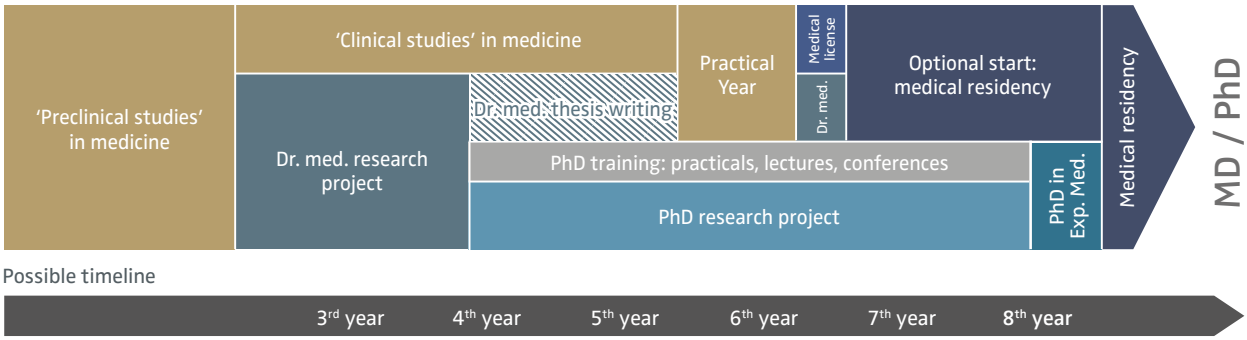
MOLECULAR MEDICINE (BACHELOR'S COURSE)

Teaching the theoretical and practical skills in the basic fields of imaging science, including MRI, PET, CT, SPECT, OI and multimodal imaging, is one main focus of this module. We also impart knowledge regarding imaging-related basic physical principles and the various applications currently used in radiology and biomedical science. Finally, we offer insight into the complex and therefore challenging (but nevertheless fascinating) and forward-looking field of pharmacokinetic modeling using imaging data.

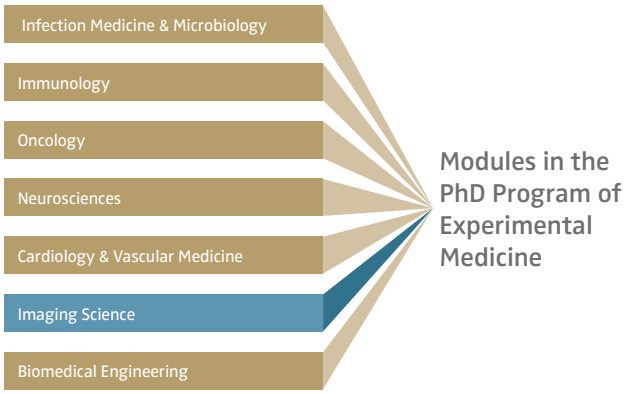
Teaching language: English

PHD EXPERIMENTAL MEDICINE

Multidisciplinary training within the PhD program of Experimental Medicine is offered by representatives and experienced teaching staff from different research areas within the Faculty of Medicine in Tübingen. This elective program involves innovative techniques, such as problem-based learning courses, case studies, lab rotations and intensive training in the stimulating field of preclinical imaging sciences. This module is further strengthened by the diverse networks between many different research areas and by having ac-



The Eberhard Karls University Tübingen specializes in a number of innovative fields of research:



cess to all of the state-of-the-art research facilities located in Tübingen. A special feature of this program is the possibility of receiving the double academic degree of MD/PhD. The double degree program is targeted toward students seeking a challenging, research-oriented medical education while acquiring in-depth scientific training at an early stage in their career. Dual training as a clinical resident and PhD is feasible. Further information is provided in the brochure 'PhD Program Experimental Medicine' available online (**www.medizin.uni-tuebingen.de/en-de/medizinische-fakultaet/promotionen/phd-studiengang**).
Teaching language: English

POST-GRADUATE TRAINING

Complementing the hands-on courses, we offer as part of our strong commitment to academic teaching, the Werner Siemens Imaging Center also offers a great opportunity for all postgraduate, highly motivated and excellent students from all over the world to enter into the fascinating and complex field of preclinical imaging. These outstanding young scientists can ultimately graduate with the title of either Dr. rer. nat., Dr. sc. hum., PhD, Dr. med. or Dr. med. dent. The doctoral titles are awarded by the Faculty of Science or the Faculty of Medicine.

INTERNATIONAL WORKSHOPS

A highly attended and prestigious annual Small Animal Workshop is organized by the Werner Siemens Imaging Center. This workshop provides an overview of imaging science in general, basic animal handling techniques and state-of-the-art small animal imaging modalities, including microPET, microSPECT, MRI, PET/MRI, microCT, fluorescence and bioluminescence OI, ultrasound, image analysis software solutions and basic laboratory methods (**www.isct.uni-tuebingen.de/wsic/teaching/workshops/small-animal-imaging-workshop**).
In addition, another annual and worldwide renowned PET/MRI workshop was established by the Department of Radiology several years ago, with a focus on the emerging applications of PET/MRI and implications in research and clinical practice. This workshop addresses a highly interdisciplinary audience of clinical experts and imaging advocates to initiate a lively ongoing exchange of perspectives and experiences and to foster vigorous discussions on the future of hybrid imaging technologies.

Dr. Carsten Calaminus
Head of Academic Teaching
+49-7071-29-87532
carsten.calaminus@med.uni-tuebingen.de

ADMINISTRATIVE & SCIENTIFIC MANAGEMENT

ADMINISTRATIVE MANAGEMENT

The office at the Werner Siemens Imaging Center serves as an initial point of contact for internal and external project partners and funding organizations. The office ensures good day-to-day internal and external communication and correspondence with partners and external bodies. The office is further responsible for organizing project meetings, executing research agreements with industry partners, monitoring of contractual duties and supervising intellectual property rights and patent management.

SCIENTIFIC COORDINATION

The scientific coordination division of the office at the Werner Siemens Imaging Center is responsible for overall scientific coordination of research activities and monitoring of progress to ensure the fulfillment of all project aims and objectives. Additional tasks include the guidance of compliance with ethics and research integrity requirements as well as the coordination of timely submission of reports, deliverables or periodic technical reports at the requested level of scientific quality. In addition, we work with media and administration offices to present the department and its research activities. The scientific coordination office also supports Platform II Medical Technology of the University's Excellence Strategy. Our aim is to stimulate an open exchange of ideas between the university and the university hospital, thus facilitating competent communication with various stakeholders and academic and industrial partners to maintain cutting-edge research.

FUND MANAGEMENT

The financial management of the office at the Werner Siemens Imaging Center is responsible for overall budget management as well as financial and administrative management of third-party funds from funding organizations such as the European Union, the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG), the Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF), the National Institutes of Health (NIH) or several foundations (Werner Siemens-Stiftung, Michael J. Fox Foundation, Adolf-Leuze-Stiftung and Dr. K. H. Eberle Stiftung, among others). Tasks include monitoring correct budgeting (according to the rules outlined by the funding organizations) and cost justification during the application phase and during the project duration as well as the preparation of financial audits and the guidance of partners on financial rules and requirements.

Dr. Rebecca Rock
Head of Office
+49-7071-29-83450
rebecca.rock@med.uni-tuebingen.de

Ines Herbon
Personal Assistant
+49-7071-29-87443
ines.herbon@med.uni-tuebingen.de

Dr. Neele Hübner
Scientific Coordination
+49-7071-29-83459
neele.huebner@med.uni-tuebingen.de

Dr. Andreas Dieterich
Fund Management
+49-7071-29-83459
andreas.dieterich@med.uni-tuebingen.de

05

IMPORTANT PUBLICATIONS

Henneberg S, Hasenberg A, Maurer A, Neumann F, Bornemann L, Gonzalez-Menendez I, Kraus A, Hasenberg M, Thornton CR, Pichler BJ, Gunzer M, Beziere N. Antibody-guided *in vivo* imaging of Aspergillus fumigatus lung infections during anti-fungal azole treatment.
Nat Commun. 2021 Mar 17;12(1):1707.

Castaneda-Vega S, Katiyar P, Russo F, Patzwaldt K, Schnabel L, Mathes S, Hempel JM, Kohlhofer U, Gonzalez-Menendez I, Quintanilla-Martinez L, Ziemann U, la Fougere C, Ernemann U, Pichler BJ, Disselhorst JA, Poli S. Machine learning identifies stroke features between species.
Theranostics. 2021 Jan 1;11(6):3017-3034.

Fischer K, Fenzl A, Liu D, Dyar KA, Kleinert M, Brielmeier M, Clemmensen C, Fedl A, Finan B, Gessner A, Jastroch M, Huang J, Keipert S, Klingenspor M, Brüning JC, Kneilling M, Maier FC, Othman AE, Pichler BJ, Pramme-Steinwachs I, Sachs S, Scheideler A, Thaiss WM, Uhlenhaut H, Ussar S, Woods SC, Zorn J, Stemmer K, Collins S, Diaz-Meco M, Moscat J, Tschöp MH, Müller TD. The scaffold protein p62 regulates adaptive thermogenesis through ATF2 nuclear target activation.
Nat Commun. 2020 May 8;11(1):2306.

Brenner E, Schörg BF, Ahmetlić F, Wieder T, Hilke FJ, Simon N, Schroeder C, Demidov G, Riedel T, Fehrenbacher B, Schaller M, Forschner A, Eigentler T, Niessner H, Sinnberg T, Böhm KS, Hömberg N, Braumüller H, Dauch D, Zwirner S, Zender L, Sonanini D, Geishauser A, Bauer J, Eichner M, Jarick KJ, Beilhack A, Biskup S, Döcker D, Schadendorf D, Quintanilla-Martinez L, Pichler BJ, Kneilling M, Mocikat R, Röcken M. Cancer immune control needs senescence induction by interferon-dependent cell cycle regulator pathways in tumours.
Nat Commun. 2020 Mar 12;11(1):1335.

Mannheim JG, Mamach M, Reder S, Traxl A, Mucha N, Disselhorst JA, Mittelhäuser M, Kuntner C, Thackeray JT, Ziegler S, Wanek T, Bankstahl JP, Pichler BJ. Reproducibility and comparability of preclinical PET imaging data: A multi-center small animal PET study.
J Nucl Med. 2019 Oct;60(10):1483-1491.

Hoffmann SHL, Reck DI, Maurer A, Fehrenbacher B, Sceneay JE, Poxleitner M, Oez HH, Ehrlichmann W, Reischl G, Fuchs K, Schaller M, Hartl D, Kneilling M, Moeller A, Pichler BJ, Griessinger CM. Visualization and quantification of *in vivo* homing kinetics of myeloid- derived suppressor cells in primary and metastatic cancer.
Theranostics. 2019 Aug 12;9(20):5869-5885.

Beziere N, Fuchs K, Maurer A, Reischll G, Brueck J, Ghoreschi K, Fehrenbacher B, Berrio DC, Schenke-Layland K, Kohlhofer U, Quintanilla-Martinez L, Gawaz M, Knellling M, Pichler B. Imaging fibrosis in inflammatory diseases: targeting the exposed extracellular matrix.
Theranostics. 2019 Apr 13;9(10):2868-2881.

Disselhorst JA, Krueger MA, Ud-Dean SMM, Bezrukov I, Jarboui MA, Trautwein C, Traube A, Spindler C, Cotton JM, Leibfritz D, Pichler BJ. Linking imaging to omics utilizing image-guided tissue extraction.
Proc Natl Acad Sci U S A. 2018 Mar 27;115(13):E2980-E2987.

Thunemann M, Schörg BF, Feil S, Lin Y, Voelkl J, Golla M, Vachaviolos A, Kohlhofer U, Quintanilla- Martinez L, Olbrich M, Ehrlichmann W, Reischl G, Griessinger CM, Langer HF, Gawaz M, Lang F, Schäfers M, Kneilling M, Pichler BJ, Feil R. Cre/lox-assisted non-invasive *in vivo* tracking of specific cell populations by positron emission tomography.
Nat Commun. 2017 Sep 5;8(1):444.

Nus M, Sage AP, Lu Y, Masters L, Lam BYH, Newland S, Weller S, Tsiantoulas D, Raffort J, Marcus D, Finigan A, Kitt L, Figg N, Schirmbeck R, Kneilling M, Yeo GSH, Binder CJ, de la Pompa JL, Mallat Z. Marginal zone B cells control the response of follicular helper T cells to a high-cholesterol diet.
Nat Med. 2017 May;23(5):601-610.

Schmitz J, Schwab J, Schwenck J, Chen Q, Quintanilla-Martinez L, Hahn M, Wietek B, Schwenzer N, Staebler A, Kohlhofer U, Aina OH, Hubbard NE, Reischl G, Borowsky AD, Brucker S, Nikolaou K, La Fougère C, Cardiff RD, Pichler BJ, Schmid AM. Decoding intratumoral heterogeneity of breast cancer by multiparametric *in vivo* imaging: A translational study.
Cancer Res. 2016 Sep 15;76(18):5512-22.

Rolle AM, Hasenberg M, Thornton CR, Solouk-Saran D, Männ L, Weski J, Maurer A, Fischer E, Spycher PR, Schibli R, Boschetti F, Stegemann-Koniszewski S, Bruder D, Severin GW, Autenrieth SE, Krappmann S, Davies G, Pichler BJ, Gunzer M, Wiehr S. ImmunoPET/MR imaging allows specific detection of Aspergillus fumigatus lung infection *in vivo*.
Proc Natl Acad Sci U S A. 2016 Feb 23;113(8):E1026-33.

Kolb A, Sauter AW, Eriksson L, Vandenbrouke A, Liu CC, Levin C, Pichler BJ, Rafecas M. Shine-Through in PET/MR Imaging: Effects of the Magnetic Field on Positron Range and Subsequent Image Artifacts.
J Nucl Med. 2015 Jun;56(6):951-4.

Griessinger CM, Maurer A, Kesenheimer C, Kehlbach R, Reischl G, Ehrlichmann W, Bukala D, Harant M, Cay F, Brück J, Nordin R, Kohlhofer U, Rammensee HG, Quintanilla-Martinez L, Schaller M, Röcken M, Pichler BJ, Kneilling M. ^{64}Cu antibody-targeting of the T-cell receptor and subsequent internalization enables *in vivo* tracking of lymphocytes by PET.
Proc Natl Acad Sci USA. 2015 Jan 27;112(4):1161-6.

Maier FC, Wehrl HF, Schmid AM, Mannheim JG, Wiehr S, Lerdkrai C, Calaminus C, Stahlschmidt A, Ye L, Burnet M, Stiller D, Sabri O, Reischl G, Staufenbiel M, Garaschuk O, Jucker M, Pichler BJ. Longitudinal PET-MRI reveals β-amyloid deposition and rCBF dynamics and connects vascular amyloidosis to quantitative loss of perfusion.
Nat Med. 2014 Dec;20(12):1485-92.

Kolb A, Parl C, Mantlik F, Liu CC, Lorenz E, Renker D, Pichler BJ. Development of a novel depth of interaction PET detector using highly multiplexed G-APD cross-strip encoding.
Med Phys. 2014 Aug;41(8):081916.

Wehrl HF, Hossain M, Lankes K, Liu CC, Bezrukov I, Martirosian P, Schick F, Reischl G, Pichler BJ. Simultaneous PET-MRI reveals brain function in activated and resting state on metabolic, hemodynamic and multiple temporal scales.
Nat Med. 2013 Sep;19(9):1184-9.

Rensch C, Waengler B, Yaroshenko A, Samper V, Baller M, Heumesser N, Ulin J, Riese S, Reischl G. Microfluidic reactor geometries for radiolysis reduction in radiopharmaceuticals.
Appl Radiat Isot. 2012 Aug;70(8):1691-7.

Maetzler W, Reimold M, Schittenhelm J, Vorgerd M, Bornemann A, Kotter I, Pfannenberg C, Reischl G, Schols L. Increased [^{11}C]PIB-PET levels in inclusion body myositis are indicative of amyloid beta deposition.
J Neurol Neurosurg Psychiatry. 2011 Sep;82(9):1060-2.

Sauter AW, Wehrl HF, Kolb A, Judenhofer MS, Pichler BJ. Combined PET/MRI: one step further in multimodality imaging.
Trends Mol Med. 2010 Nov;16(11):508-15.

Pichler BJ, Kolb A, Nägele T, Schlemmer HP. PET/MRI: paving the way for the next generation of clinical multimodality imaging applications.
J Nucl Med. 2010 Mar;51(3):333-6.

Kneilling M , Mailhammer R, Hültner L, Schönberger T, Fuchs K, Schaller M, Bukala D, Massberg S, Sander CA, Eichner M, Maier KL, Hallmann R, Pichler BJ, Haubner R, Gawaz M, Pfeffer K, Biedermann T, Röcken M. Direct crosstalk between mast cell-TNF and TNFR1-expressing endothelia mediates local tissue inflammation.
Blood. 2009 Aug 20;114(8):1696-706.

Judenhofer MS, Wehrl HF, Newport DF, Catana C, Siegel SB, Becker M, Thielscher A, Kneilling M, Lichy M, Eichner M, Klingel K, Reischl G, Widmaier S, Röcken M, Nutt RE, Machulla HJ, Uludag K, Cherry SR, Claussen CD, Pichler BJ. Simultaneous PET-MRI: a new approach for functional and morphological imaging.
Nat Med. 2008 Apr;14(4):459-65.

06

LOCATION

SCIENTIFIC ENVIRONMENT & CULTURE: THE TÜBINGEN RESEARCH CAMPUS

Tübingen is a traditional, historic university town located on the Neckar River, 40 km southwest of Stuttgart on the fringe of the Swabian Jura Mountains and the Black Forest. The city first appeared in official records in 1191, but Tübingen's castle dates back to 1078. The Eberhard Karls University Tübingen is one of Germany's oldest universities and is internationally recognized for medicine, theological sciences and the humanities. It was founded in 1477 by Count Eberhard V.

The University of Tübingen is well known for its eminent and leading international researchers, past and present. These researchers include, for example, Johannes Friedrich Miescher, a Swiss physician and biologist who isolated nucleic acids for the first time at the University of Tübingen and paved the way to the discovery of deoxyribonucleic acid (DNA); Georg Wilhelm Friedrich Hegel, a German philosopher who revolutionized the philosophical community with his idealistic views; and Johannes Kepler, a German astronomer who is renowned for defining the three laws of planetary motion.

Since the beginning of the 20th century, scientists at the University of Tübingen have recovered and studied the oldest evidence of figurative art, music and religious beliefs of mankind in caves in the Swabian Alb. The Museum Ancient Cultures at Hohentübingen Castle, part of the Museum of the University of Tübingen, houses the largest collection of original discoveries from the Paleolithic period, including the famous 40,000-year-old horse made of mammoth ivory from the Vogelherd Cave.

© Museum of the University of Tübingen

Eberhard Karls University Tübingen – A German University of Excellence with three Clusters of Excellence funded by the Excellence Strategy of the German federal and state governments.

- Controlling Microbes to Fight Infections (CMFI)
- Image-Guided and Functionally Instructed Tumor Therapies (iFIT)
- Machine Learning: New Perspectives for Science (ML)

Interdisciplinary interactions between various research areas of the University of Tübingen are implemented via four research platforms:

- Clinical Research and Drug Discovery & Development
- Medical Technology
- Environmental Systems
- Global Encounters

The main research focus at the Faculty of Medicine is currently on these areas:

- Neuroscience
- Immunology and Oncology
- Infection Research
- Diabetes and Vascular Medicine

SCIENTIFIC ENVIRONMENT

In addition to the university with its 7 faculties, Tübingen also has 17 hospitals affiliated with the University's Faculty of Medicine. As a result of the third party funds acquired, the number of Collaborative Research Centers, Graduate Programs, Research Groups and involvement in national and international collaborations, the Faculty of Medicine in Tübingen is rated as one of the top ten Faculties of Medicine in all of Germany's accepted ranking lists.

Tübingen offers a unique scientific environment and hosts institutions such as the Hertie Institute for Clinical Brain Research (HIH), which was established in Tübingen with promotional funds from the charitable Hertie Foundation. As a result of its close integration with the Department of Neurology and hence with the Center for Neurology, it enables optimal coordination between basic research and medical applications.

Close collaborations exist between the University and the Max Planck Institutes located in Tübingen:

- Max Planck Institute for Biological Cybernetics
- Max Planck Institute for Developmental Biology
- Friedrich Miescher Laboratory

THE TOWN OF TÜBINGEN AND SURROUNDING AREA

Currently, Tübingen is a small, typical German university town with 91,000 inhabitants and 27,000 students, making Tübingen a city with a very young average population in Germany. Life in the city is dominated by its many students, combining the character of a lovingly restored medieval town center with the colorful bustle and typical atmosphere of a young, cosmopolitan student town.

The active cultural scene offers events, museum exhibitions and collections, festivals, concerts, stage plays and readings by poets of international reputation.

Numerous parks, gardens, and forested areas invite exploration by foot or bicycle. The immediate surroundings of the town provide an outstanding environment for outdoor activities such as swimming, cycling, hiking and cross-country skiing in the winter.

> www.tuebingen.de

STUTTGART

The closest major city to Tübingen is Stuttgart, which is located 40 km northeast of Tübingen. Stuttgart, the capital of Baden-Württemberg, provides all of the shopping facilities and cultural lifestyle of a large city. Stuttgart has a wide range of cultural offerings, including several museums, theaters and an opera house.

> www.stuttgart-tourist.de

The city of Stuttgart

Schlossplatz in the center of the city

© Stuttgart-Marketing GmbH

SWABIAN MOUNTAINS (SCHWÄBISCHE ALB)

The Swabian Mountains, a high plateau with the highest mountain (Lemberg) reaching 1,015 m, are located close to Tübingen. The spectacular landscape and magnificent natural environment make the Swabian Mountains an attractive destination for hiking and cycling. The numerous castles, churches and monasteries as well as caves and sites of important fossil discoveries and prehistorical findings are also worth visiting.

> www.schwaebischealb.de

Castle Hohenzollern near Hechingen

Albtrauf near Mössingen

© Schwäbische Alb Tourismusverband e.V.

BLACK FOREST (SCHWARZWALD)

The Black Forest begins approximately 40 km west of Tübingen and offers several opportunities for sporting activities, such as hiking and cycling, as well as great country sides and cities that are worth visiting. With mountains reaching 1,493 m (Feldberg) in height, the Black Forest is also a popular skiing region in the winter.

> www.blackforest-tourism.com

Gütenbach near Freiburg

Winter landscape near Schluchsee

© Schwarzwald Tourismus

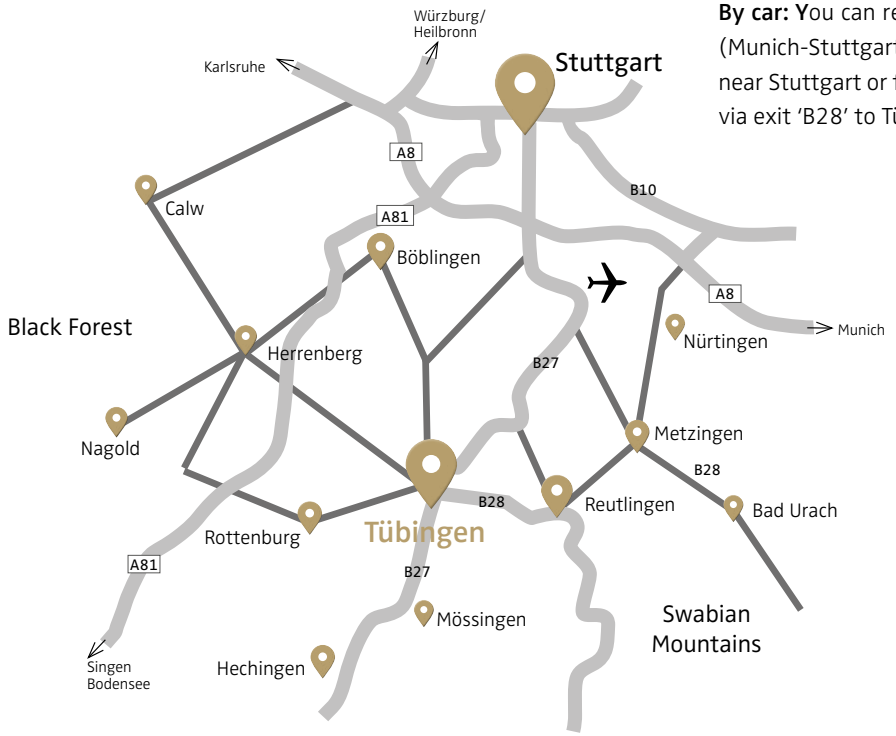
HOW TO REACH US

As a result of the close proximity to Stuttgart, Tübingen is easily reached by plane. Stuttgart has a modern international airport with nonstop flights to and from major international destinations.

By plane: The nearest international airport is Stuttgart (code: STR). From the airport, you can reach our laboratory within 20 min by car or taxi. Public transportation by bus or train is also available.

By train: You can reach our laboratory from Tübingen main station within 10 min by taxi or within 7 min by riding the No. 5 bus line to the 'Uni-Kliniken Tal' stop.

By car: You can reach our laboratory from the autobahn A8 (Munich-Stuttgart or Karlsruhe-Stuttgart) via the exit 'B27' near Stuttgart or from the autobahn A81 (Stuttgart-Singen) via exit 'B28' to Tübingen.



07

CONTACT INFORMATION

CONTACT INFORMATION

Werner Siemens Imaging Center
Department of Preclinical Imaging and Radiopharmacy
Eberhard Karls University Tübingen
Röntgenweg 13, 72076 Tübingen, Germany

Phone: +49 7071 29-87443
Fax: +49 7071 29-4451
E-mail: office.WSIC@med.uni-tuebingen.de
Web: wsic.isct.uni-tuebingen.de

Prof. Dr. Bernd Pichler
Chair & Director
+49-7071-29-83427
bernd.pichler@med.uni-tuebingen.de

Dr. Julia Mannheim
Deputy Head of Department
+49-7071-29-82974
julia.mannheim@med.uni-tuebingen.de

Ines Herbon
Personal Assistant
+49-7071-29-87443
ines.herbon@med.uni-tuebingen.de

Dr. Rebecca Rock
Head of Office
+49-7071-29-83450
rebecca.rock@med.uni-tuebingen.de

Funda Cay
Leading Technologist
+49-7071-29-87507
funda.cay@med.uni-tuebingen.de

Dr. Carsten Calaminus
Academic Teaching; Animal Services
+49-7071-29-87532
carsten.calaminus@med.uni-tuebingen.de

Dr. Marcel Krüger
Industrial Cooperation
+49-7071-29-82972
marcel.krueger@med.uni-tuebingen.de

Hans Jörg Rahm
IT & Radiopharmacy Controlling
+49-7071-29-80539
hans.rahm@med.uni-tuebingen.de

RESEARCH GROUP LEADERS

Dr. Nicolas Bézière
Group Leader Infection & Inflammation
+49-7071-29-87511
nicolas.beziere@med.uni-tuebingen.de

Dr. Salvadore Castaneda Vega
Group Leader Translational Neuroimaging
+49-7071-29-87479
salvador.castaneda@med.uni-tuebingen.de

Prof. Dr. Kristina Herfert
Head of Functional
and Metabolic Brain Imaging
+49-7071-29-87680
kristina.herfert@med.uni-tuebingen.de

Dr. Manfred Kneilling
Group Leader Immunology & Inflammation
+49-7071-29- 86870
manfred.kneilling@med.uni-tuebingen.de

Dr. Marcel Krüger
Group Leader Oncology
+49-7071-29-82972
marcel.krueger@med.uni-tuebingen.de

Dr. Julia Mannheim
Group Leader PET & Multimodal
Imaging Science
+49-7071-29-82974
julia.mannheim@med.uni-tuebingen.de

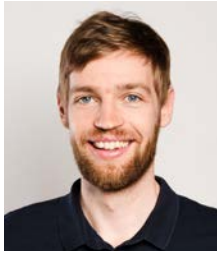
Prof. Dr. André F. Martins
Head of Advanced Preclinical
Metabolic Imaging and Cell Engineering
+49-7071-29-87487
andre.martins@med.uni-tuebingen.de

Dr. Andreas Maurer
Group Leader Imaging Probe Development
+49-7071-29-87490
andreas.maurer@med.uni-tuebingen.de

Apl. Prof. Dr. Gerald Reischl
Head of Radiopharmacy
+49-7071-29-80531
gerald.reischl@uni-tuebingen.de

Dr. Andreas Schmid
Group Leader MR & Multimodal Imaging Science
+49-7071-29-87510
a.schmid@med.uni-tuebingen.de

Dr. Fabian Schmidt
Group Leader Imaging Detector Physics
+49-7071-29-87481
f.schmidt@med.uni-tuebingen.de



Dr. Johannes Schwenck, MD, PhD
Group leader Translational and Preclinical Imaging in Inflammation & Cancer
+49-7071-29-86870
johannes.schwenck@med.uni-tuebingen.de

Dr. Dominik Sonanini
Group Leader Translational Immuno-imaging & Cancer Immunotherapies
+49-7071-29-87589
dominik.sonanini@med.uni-tuebingen.de

Dr. Christoph Trautwein
Group Leader Metabolomics & Systems Medicine
+49-7071-29-83426
christoph.trautwein@med.uni-tuebingen.de

Prof. Dr. Bettina Weigelin
Head of Preclinical Imaging of the Immune System
+49-7071-29-87414
bettina.weigelin@med.uni-tuebingen.de

Please visit our website www.isct.uni-tuebingen.de/wsic to download a digital version of this brochure.

IMPRINT

PUBLISHER

University of Tübingen
Department of Preclinical Imaging and Radiopharmacy
Werner Siemens Imaging Center
Röntgenweg 11-17
72076 Tübingen
GERMANY
Phone: +49-7071-29-87443
Fax: +49-7071-29-4451
Email: office.WSIC@med.uni-tuebingen.de
www.isct.uni-tuebingen.de/wsic

EDITOR

Dr. Neele Hübner, Dr. Julia Mannheim, Prof. Dr. Bernd Pichler (responsible)

LAYOUT

neunpunktzwei Werbeagentur GmbH
Bahnhofstraße 26
72138 Kirchentellinsfurt
www.neunpunktzwei.de

PRINT

Druckerei Sautter GmbH
Röntgenstraße 24
72770 Reutlingen
www.sautter.de

COPYRIGHTS

inga pass photography (ingapaas.de): laboratory pictures
pixelcatcher (pixelcatcher.de): laboratory pictures, portrait pictures, group picture (p. 59)
Beate Armbruster Fotografie (beatearmbruster.de): group picture (p. 15)

Effective: November 2021

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Eberhard Karls University Tübingen

Werner Siemens Imaging Center
Department of Preclinical Imaging and Radiopharmacy
Röntgenweg 13 · 72076 Tübingen · Germany
www.isct.uni-tuebingen.de/wsic