Frauke Alves[a,b,c], Christian Dullin[a,c], Claus Feldmann[d], Andrea Markus[a] http://dx.medra.org/10.17374/CI.2026.108.3.9
[a]Max-Planck-Institute for Multidisciplinary Sciences (MPI-NAT)
Translational Molecular Imaging, Goettingen (D)
markus@mpinat.mpg.de
[b]University Medical Center Goettingen (UMG)
Translational Molecular Imaging, Clinic for Haematology and Medical Oncology - Goettingen (D)
falves@gwdg.de
[c]University Medical Center Goettingen (UMG)
Translational Molecular Imaging, Clinical and Interventional Radiology - Goettingen (D)
christian.dullin@med.uni-goettingen.de
[d]Institute of Inorganic Chemistry, Karlsruhe Institute of Technology (KIT) - Karlsruhe (D)
claus.feldmann@kit.edu

MULTIMODAL IMAGING FOR THERAPY AND DIAGNOSIS

A cross-scale multimodal imaging platform has been developed to assess nanoparticle (NP)-based therapeutic and diagnostic concepts. In vivo distribution, and intracellular trafficking of fluorescent NP are monitored by optical imaging and therapy response is evaluated by computer tomography (CT). Correlative micro-CT, light-sheet- and multiphoton microscopy of tissue have been optimized for disease subclassification.

Introduction

The number of cancer cases is increasing worldwide, and cancer remains one of the most common causes of death. In a variety of aggressive, heterogeneous solid tumors (*e.g.*, pancreatic ductal adenocarcinoma/PDAC, colorectal cancer/CRC), the current chemo- and radiotherapies are largely ineffective. Moreover, resistant tumor phenotypes develop and severe side effects occur. Especially in the context of personalized medicine, there is an urgent need for

i) novel therapeutic strategies overcoming chemoresistance and side effects;
ii) predictive imaging biomarkers that could assist the selection of the most effective therapy.

Here, we report on the implementation of a multimodal imaging platform across scales (Fig. 1) to monitor novel NP-based therapeutic concepts.

Non-invasive computer tomography (CT) is applied to assess the therapeutic response with respect to anatomical alteration, *e.g.* tumor growth as well as lung function (LF). Optical imaging is the main modality to monitor the *in vivo* distribution, and intracellular trafficking of near infrared fluorescent (NIRF) labeled NP. Light-sheet- (LSM) and multiphoton microscopy (MPM) are optimized to assess NP distribution in tissues or Patient Derived Organoids (PDOs), and together with micro-CT to characterize tissue biopsies for cancer subclassification by correlative imaging to allocate patients to the best therapy.

Nanoparticle-based novel therapeutic approaches

NP, up to 100 nm in diameter and made from a large variety of materials, are widely used for cancer therapy, since they are known to accumulate at the site of the tumor due to the enhanced permeability and retention effect. Understanding their cellular uptake, drug release, biodistribution, accumulation and mechanisms for heterogeneous distribution in healthy organs and tumors is a key to their successful clinical application. Here, multimodal imaging across scales is shown to be a powerful tool for non-invasive visualization of NP in preclinical tumor models as well as with high spatial and temporal resolution to assess the distribution of NP in cancer tissue on (sub-) cellular scales.

For effective chemotherapy with less side effects, we use hybrid NP and core@shell NP as nanocarriers for improved treatment. Two groups of zirconyl-containing NP were tested:

i) so-called inorganic-organic hybrid NP containing a high load of the chemotherapeutic agents gemcitabine [1], SN38 or 5-fluorouracil [2, 3];
ii) core-shell NP as synergistic multi-drug treatment [4], for instance, with a particle core of irinotecan (ITC) covered by a particle shell of fludarabine.

Some NP were functionalized with a clinically used antibody targeting epidermal growth factor receptor. Both NP groups showed cytotoxic activity and

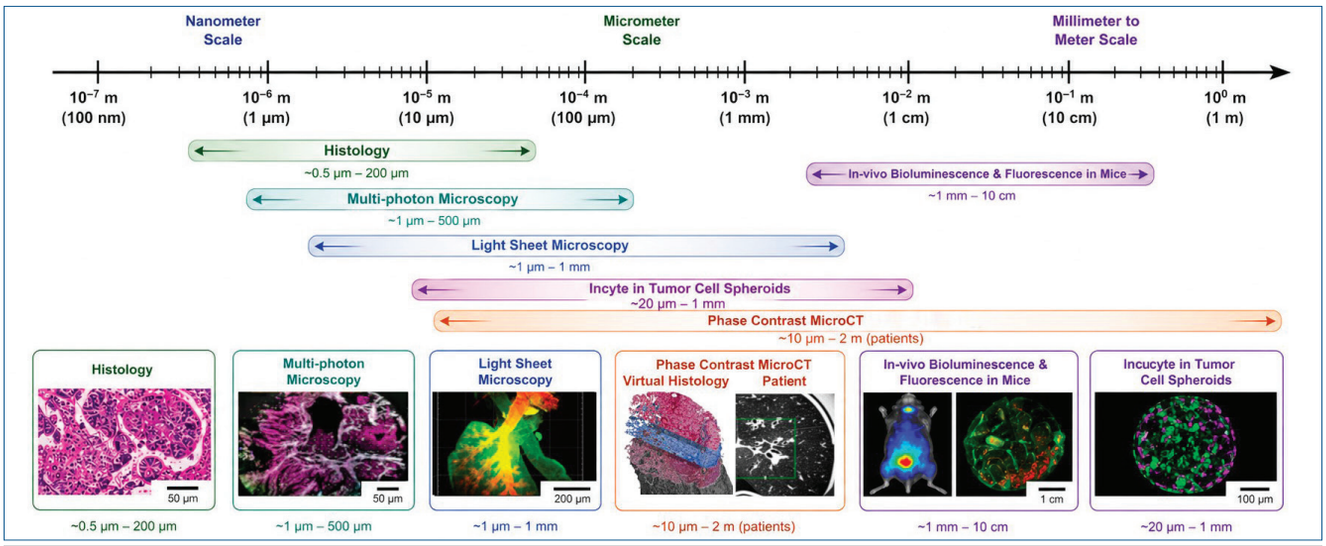

Fig. 1 - Multimodal, cross-scale and correlative imaging platform. It consists of high-resolution 3D microscopy *e.g.* light sheet (LSM) and multi photon microscopy (MPM), phase contrast CT based correlative histology, OI (Bioluminescence- and near infrared fluorescence imaging), as well as live cell imaging (Incucyte® Live-Cell Analysis System), thereby bridging a resolution range from sub-micrometer to several cm. Representative images of the different imaging modalities are shown

cellular uptake in relevant PDAC- and CRC disease models [1, 4]. Incorporating a fluorescent dye in the NP allowed fluorescence-based tracking *in vitro*, especially tumor cell uptake (Fig. 2) and *in vivo*, tracking in mice. Selective delivery of extraordinarily high concentrations of already phosphorylated gemcitabine monophosphate (GMP) to the primary tumor and metastatic sites via the hybrid NP was confirmed in PDAC-bearing mice. Tumor accumulation was visualized by fluorescence-labeled NP in combination with optical imaging (OI). The anti-tumor efficacy was determined by tumor reduction in response to already phosphorylated, activated GemP-hybrid NP (76 wt-% of total NP mass) [1]. Moreover, we monitored NP dissolution [5] and intracellular pH in tumor cells [6] by incorporating activatable dyes as sensors. In a single core@shell NP, we combined lipophilic Irinotecan (ITC, 22wt% of total NP mass) and hydrophilic fluoro-2'-deoxyuridine-5'-phosphate (10wt% FdUMP of total NP mass), equivalent to drug concentrations used in clinically applied cocktails of free drugs (FOLFIRI) to treat CRC. ITC-FdUMP-NP demonstrate equivalent cytotoxic effects to the free drugs in rectal cancer PDOs with a sustained drug-release profile [7]. Achieving even a higher drug loading with lipophilic and hydrophilic drugs of 57wt% of total NP mass, we demonstrate that these ITC-FdUMP-nanocarriers, in contrast to free drugs, induce a delayed but substantially prolonged cytotoxic response in CRC cell lines. By incorporating functional fluorescent dyes, we analyzed intracellular trafficking and drug release of the core@shell NP that directly links NP design to their extended therapeutic window, with the potential to reduce the systemic side effects. We found that after slow cellular uptake predominantly via macropinocytosis, the NP rapidly traffic to endolysosomal compartments, where the acidic environment induces sustained drug release [8].

To overcome the immune suppression in PDAC, a mesothelin peptide-formulated nanovaccine was applied consisting of NP that contain immunomodulating components intended to induce specific T cell responses. The encapsulation of peptides and adjuvants into NP facilitates the delivery of vaccine compunds and preserves them from rapid degradation at the same time avoiding a local immune response. The incorporation of fluorescent dyes into the nanovaccine formulation allows visualization of their intracellular uptake in antigen-presenting cells of lymph nodes by fluorescence microscopy [9]. Nanovaccine re-stimulation of splenocytes from vaccinated mice elevated interferon-γ levels *in vitro* compared to controls, and serum analysis revealed increased MSLN-specific IgM and IgG antibodies *in vivo*. Prophylactic and early therapeutic vaccination inhibited KPC cell induced tumor progression *in vivo* and enhanced CD8+ T cell tumor infiltration [9].

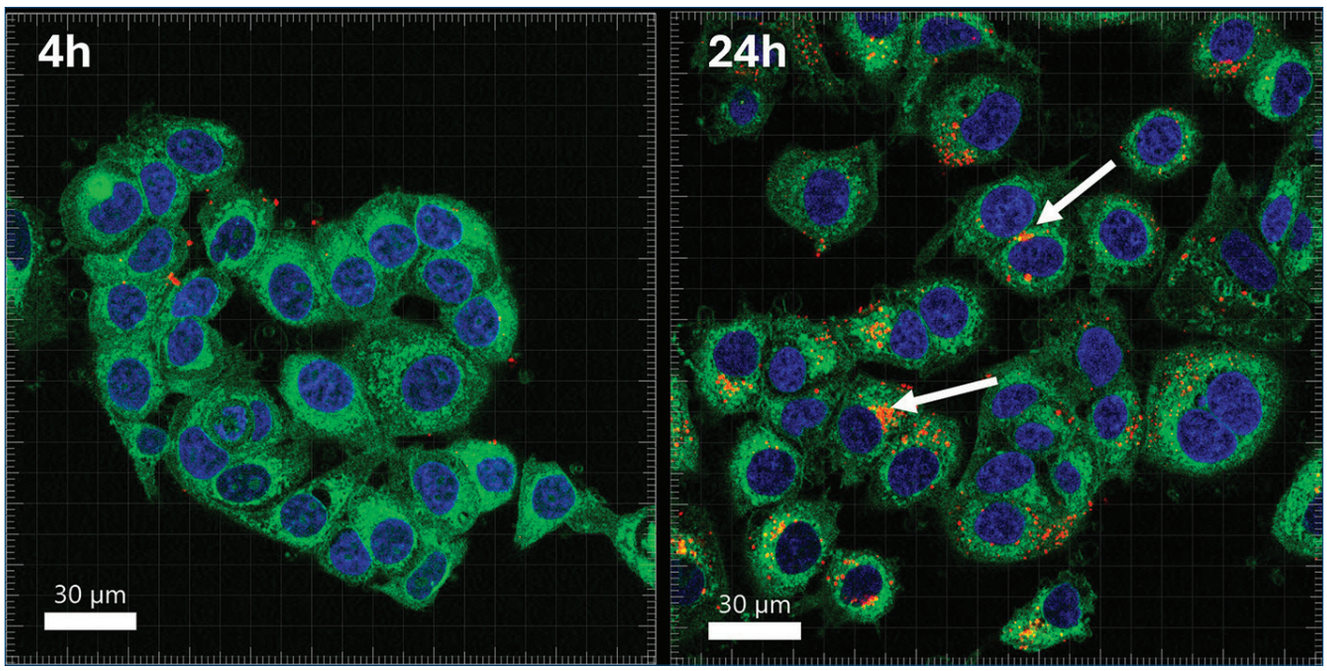

Fig. 2 - Intracellular uptake of nanoparticles. Human MCF7 breast tumor cells were incubated 4 h or 24 h with therapeutic core@ shell NP (red). The cells have taken up significantly more NP at 24 h than at 4 h of incubation. Lysosomes were stained with Lysotracker green and the nuclei were counterstained with Hoechst 33342 (blue). Arrows indicate colocalization (orange colour) of NP with stained lysosomes. Scale bar = 30 µm

For *in vitro* validation of immunotherapy, we developed an AI-driven platform, Curated Cell Life Imaging Dataset (OrganoIDNetData) [10]. Analysis of live-cell imaging data from PDAC PDOs, cultured either alone or with human peripheral blood mononuclear cells, enables real-time, personalized assessment of therapeutic responses [11]. Using an optimized "sandwich" protocol, we maintained PDO spatial organization and established stable conditions for long-term live imaging, reproducible and applicable to co-cultures of CAR T cells and PDAC PDOs. We implemented an *in vitro* model using the mesothelin-targeted nanovaccine to stimulate PDAC-specific T cells. When co-cultured with PDAC PDOs in an organ-on-chip system, we observed enhanced T cell infiltration in mesothelin+ PDAC PDOs compared to mesothelin-KO controls, showing nanovaccine specificity and therapeutic response. These human organoid-immune cell co-cultures have the potential to assess complex processes related to nanovaccine strategies representing an optimal model for testing personalized treatment strategies [12].

Multimodal imaging towards clinical application

To better sub-classify tumor patients and thus assign optimal treatment choices, a panel of high-resolution and correlative imaging strategies are implemented for characterization of FFPE (formalin-fixed and paraffin-embedded) tumor biopsies (Fig. 1) [13]. Optical clearing methods are used to significantly enhance deep tissue visualization, providing new layers of 3D information about cellular details within cancer biopsies [14, 15]. For a comprehensive analysis, the obtained 3D imaging data from PCT, LSM, and MPM are correlated to histology, clinical data and molecular signatures. The next step will be to develop a strategy to register all 3D imaging data.
Two photon-microscopy (2PM) is applied to characterize cancer tissue in 3D, for example ECM components in biopsies of a CC patient cohort, distinguishing left- (LSCC), with higher collagen content, and right-sided colon cancer (RSCC). We show that fibers inside of tumors exhibit loss of organization, mainly in RSCC [16]. By integrating imaging features from 2PM and histology with proteomics signatures obtained from Matrix-assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI MSI), structural coherence of collagen fibers and the nuclei distribution profile of tumor tissue were correlated with the peptide signatures, offering insights into the CC proteomic landscape [17]. In addition, for label-free volumetric quantification and characteri-

zation, 2PM was applied to visualize second- and third-harmonic generating structures in different tissues. We show that the nonlinear capabilities of collagen, lipid droplets and myosin microarchitecture are strongly dependent on the preservation technique used [18]. In a fluorescence microscopy approach we also visualized in mouse lung, live over 3 hours, at nanometer resolution, dynamic interactions between immune and tumor cells within the TME, as well as drug transport, accumulation, and activity over time, key factors in developing novel therapies [19]. LSM is used to assess entire tumor tissue label-free in 3D, by depicting specific autofluorescence. Here, the optically cleared sample is illuminated laterally with a laser beam focused into a narrow light sheet, which is then moved vertically. Thus, the analysis of pathologically altered tissues is possible non-destructively and without staining to achieve detailed morphological insights *e.g.*, the *ex vivo* of the murine testicular tissue [20]. Phase-contrast micro-CT (PCT) is a technique that utilizes interference effects of the X-ray beam instead of its attenuation, significantly increasing soft tissue contrast. We applied PCT to visualize the tissue microarchitecture in detail, non-destructively, achieving the visualization of tissue at 2 µm resolution and allowing guided sectioning, that provided the basis for image registration [21, 22]. In addition, X-ray-based multiscale imaging was applied, in which details of a large sample are hierarchically mapped by scans of increasing resolution [23], or integrative multimodal imaging, in which 2D images of sections of the same FFPE tissue are registered three-dimensionally onto the micro-CT dataset [14, 24].
In vivo, anatomical alterations, *e.g.* mammary carcinoma growth [25] as well as lung function (LF) can be assessed by non-invasive CT. For LF we generated a setup for combined plethysmography and X-ray based LF measurement to monitor lung fibrosis and acute allergic inflammation in response to therapy *in vivo* over time [26, 27]. For translational imaging we developed PCT for lung imaging in patients by using an anthropomorphic human chest phantom for pilot studies [21, 28] as well as non-invasive optical motion tracking of the lung [29]. We established a novel fixation approach for entire human sized lungs that enables imaging under physiological conditions, hierarchical imaging with increasing spatial resolution as well as combining this with our analysis pipeline for FFPE-tissue [30].

Conclusions

In summary we established a multimodal imaging platform across scales to facilitate preclinically the development of novel NP-based cancer therapies. Moreover, correlative high resolution imaging strategies are optimized for validation in a large cohort of CRC samples for the identification of relevant imaging cancer biomarkers that could support future CRC subclassification.

Acknowledgements

This work has received funding from the European Union's Horizon 2020 research innovation program under the Marie Skłodowska-Curie grant agreements (861190-PAVE) and (857894-CAST), and Project FAIR CHARM (H2020) LEIT Information and Communication Technologies 101016457 (101016457-FAIRCHARM), by the Ministry for Science and Culture of Lower Saxony as part of the project "Agile, bio-inspired architectures" (ABA), and by the Deutsche Krebshilfe as part of the CANACO collaborative project (grant number 70114704).

REFERENCES

[1] M. Ischyropoulou, K. Sabljo *et al., Adv. Mater.*, 2023, **35**(46), 2305151.
[2] M. Khorenko, J. Pfeifer *et al., Mater. Chem. B*, 2023, **11**(16), 3635.
[3] K. Sabljo, M. Ischyropoulou *et al., Nanoscale*, 2024, **16**(31), 14853.
[4] D. Rudolph, M. Ischyropoulou *et al., Nanoscale Adv.*, 2024, **6**(3), 973.
[5] C. Ritschel, J. Napp *et al., Nanoscale*, 2022, **14**(43), 16249.
[6] K. Sabljo, J. Napp *et al., Chem. Commun.*, 2022, **58**(67), 9417.
[7] S. Notter, D. Choezom *et al., Small Sci.*, 2024, **4**(11), 2400196.
[8] D. Choezom, S. Notter *et al., Small Sci.*, 2026, **6**(1), e202500470.
[9] D.P. Ferrari, Ö. Çobanoglu *et al., Vaccines*, 2025, **13**(3), 314.
[10] A. Kulkarni, N. Ferreira *et al., Sci. Data*, 2024, **11**(1), 820.
[11] N. Ferreira, A. Kulkarni *et al., Cell. Oncol.*, 2025, **48**(1), 101.
[12] N. Ferreira, D. Agorku *et al., Adv. Sci.*, 2026, **13**(10), e15199.
[13] A. Papantonis, S. Rogalla *et al., Pathol*., 2025, **46**, 156.

[14] M.M.R. Sagar, A. Svetlove *et al., Front. Phys.*, 2024, 12.
[15] D. Pinkert-Leetsch, J. Frohn *et al., Cancer Imaging*, 2023, **23**(1), 43.
[16] B. Arora, A. Kulkarni *et al., Sci. Rep.*, 2024, **14**(1), 7510.
[17] A. Bharti, K. Ajinkya *et al., Npj Imaging*, 2024, **2**(1), 1.
[18] M.A. Markus, D.P. Ferrari *et al., Biomed. Opt. Express*, 2023, **14**(8), 3988.
[19] F. Ramos-Gomes, N. Ferreira *et al., Front. Bioeng. Biotechnol.*, 2020, 8.
[20] D. Pinkert-Leetsch, J.U. Rost *et al., J. Andrology*, 2022, **10**(8), 1660.
[21] J. Albers, W.L. Wagner *et al., Sci. Rep.*, 2023, **13**(1), 4788.
[22] J. Albers, M.A. Markus *et al., Sci. Rep.*, 2018, **8**, 7712.
[23] C. Dullin, J. Reiser *et al., Sci. Rep.*, 2025, **15**(1), 36475.
[24] J. Albers, A. Svetlove *et al., Sci. Rep.*, 2021, **11**(1), 10846.
[25] K. Jannasch, C. Dullin *et al., Int. J. Cancer*, 2009, **125**(1), 62.
[26] A. Khan, M.A. Markus *et al., Sci. Rep.*, 2023, **13**(1), 18637.
[27] M.A. Markus, J. Albers *et al., Sci. Rep.*, 2026, **16**(1), 12737.
[28] C. Dullin, W.L. Wagner *et al., Eur. Respir. J.*, 2024, **63**(3), 2301604.
[29] A. Svetlove, J. Albers *et al., Cells*, 2022, **11**(5), 918.
[30] C.V. Benke, J. Reiser *et al., Respir. Res.*, 2026, **27**(1), 105.

Imaging multimodale per terapia e diagnosi

È stata sviluppata una piattaforma di imaging multimodale su scala trasversale per valutare concetti terapeutici e diagnostici basati su nanoparticelle (NP). La distribuzione *in vivo* e il traffico intracellulare delle nanoparticelle fluorescenti vengono monitorati mediante imaging ottico, mentre la risposta terapeutica viene valutata tramite tomografia computerizzata (CT). La micro-CT correlativa, la microscopia *light-sheet* e la microscopia multifotonica dei tessuti sono ottimizzate per la sottoclassificazione delle patologie.