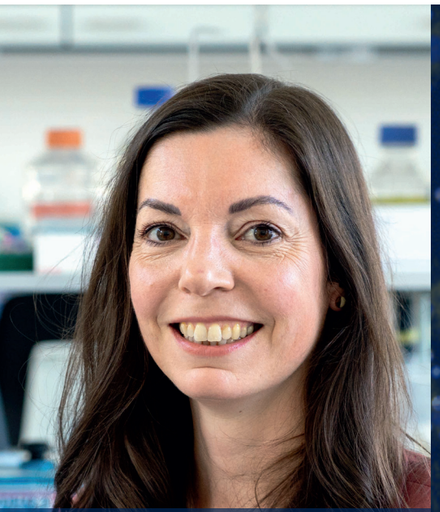
Functional Precision Oncology
Sina Oppermann



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Funktionelle Präzisionsonkologie

Funktionell-zielgerichtete Präzisionsmedizin bei Krebspatienten – von präklinischen *ex vivo* Studien zur klinischen Anwendung

Unsere Arbeitsgruppe „Functional Precision Oncology" entwickelt experimentelle und bioinformatische Ansätze, um genomische Daten durch funktionelle Analysen zu ergänzen und patientenindividuelle Tumorvulnerabilitäten zu identifizieren. 2025 konnten wir entscheidende Fortschritte bei der Etablierung einer bildbasierten ex vivo Wirkstofftest-Plattform erzielen. Mit Förderung der Rolf M. Schwiete Stiftung wurde ein Hochdurchsatz-Spinning-Disk-Mikroskop (Opera Phenix Plus, Revvity) angeschafft, das nun das Herzstück unserer Forschung bildet. Damit wurden lebendzellbasierte Fluoreszenzassays an AML-Zelllinien etabliert und erste patientenabgeleitete Modelle zur realitätsnahen Abbildung der Tumorheterogenität entwickelt. Hierbei optimieren wir verschiedenste Kulturbedingungen im 2D als auch 3D Format. Parallel entstand eine KI-gestützte Einzelzell-Analyse zur präzisen Quantifizierung zellulärer Wirkstoffantworten.

Ein erster Screen von 135 Substanzen in AML-Zelllinien identifizierte potenzielle Wirkstoff-Repositionierungen und Kombinationsstrategien zur Überwindung von Venetoclax-Resistenzen, insbesondere durch MCL-1-Hochregulation. Aktuelle Arbeiten validieren diese Befunde in primären AML-Kulturen.

Zur Integration der umfangreichen Bild- und Funktionsdaten entwickelt das Team derzeit bioinformatische Analysepipelines und KI-basierte Bildauswertungen, um prädiktive Biomarker und kombinatorische Therapiestrategien zu identifizieren.

Functional Precision Oncology

To bridge the gap between genomic knowledge and clinical application, comprehensive functional information is needed to identify tumor-specific vulnerabilities. Our group advances **functional precision oncology** by performing ***ex vivo* drug response profiling (DRP)** on **patient-derived tumor cells** to complement genomic data, particularly when genomics fail to guide therapy or tumors are driven by non-genomic factors such as the microenvironment. By providing functional data at single-cell level, we aim to 1) elucidate cancer- and drug-induced phenotypes to discover new efficacies and repositioning opportunities, 2) identify biomarkers and mechanisms underlying drug response and resistance, and 3) directly translate results into clinical application.

In 2024/2025, we achieved significant progress in establishing, standardizing, and validating image-based ex vivo drug response assays and in elucidating mechanisms of therapy resistance in acute myeloid leukemia (AML).

Establishment of predictive, image-based *ex vivo* disease models

To functionally characterize tumor-specific vulnerabilities, we are developing advanced *ex vivo* models using patient-derived AML samples from adults and children. Our goal is to generate physiologically relevant short-term cultures that maintain tumor heterogeneity and viability while enabling high-throughput drug testing. To aim this, we optimize 2D and 3D culture protocols mimicking the tumor microenvironment, including cytokine-supported co-cultures, to enhance ex vivo survival and study mechanisms of drug resistance.

With support from the Rolf M. Schwiete Foundation, we installed an automated high-throughput confocal spinning-disk microscopy system (Opera Phenix Plus, Revvity) equipped with an integrated stacker and incubator, forming the core of our imaging platform. This system is used to multiplex live-cell imaging combined with AI-assisted single-cell analysis to assess drug efficacy in AML. Using the MOLM-13 cell line, we developed a robust workflow integrating live-cell dyes (Hoechst, Annexin V, TMRE) with supervised machine learning for automated classification of viable, apoptotic, and dead cells. This scalable, phenotype-resolved 384-well Drug Response Profiling (DRP) workflow is currently being validated in primary AML samples and expanded with immunophenotypic markers to resolve cellular subpopulations and their drug sensitivities.

Functional studies on venetoclax resistance and combinatorial treatment strategies

In parallel, we investigated mechanisms of resistance to the BCL-2 inhibitor

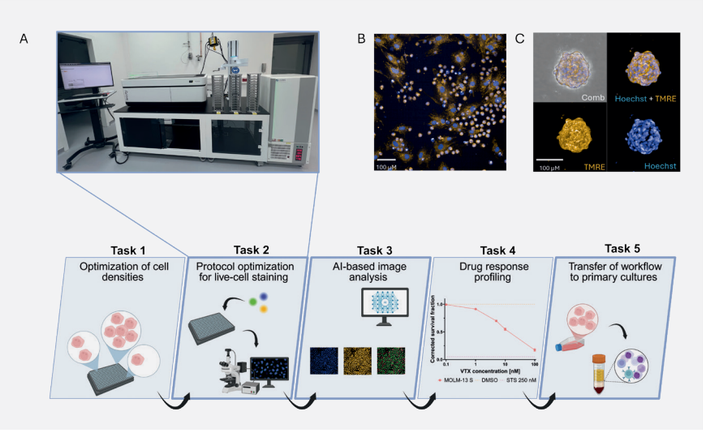

Figure 1. **Optimization and translation of an image-based workflow enabling drug response profiling in AML.** (**A**) Confocal spinning-disc microscopy (Opera Phenix Plus, Revvity). (**B**) Co-culture of MSCs and AML cells imaged by Opera Phenix; nuclei and mitochondrial potential were visualized using Hoechst (blue) and TMRE (orange), respectively. (**C**) Representative MSC spheroids imaged by Opera Phenix, stained with Hoechst (blue) and TMRE (orange).

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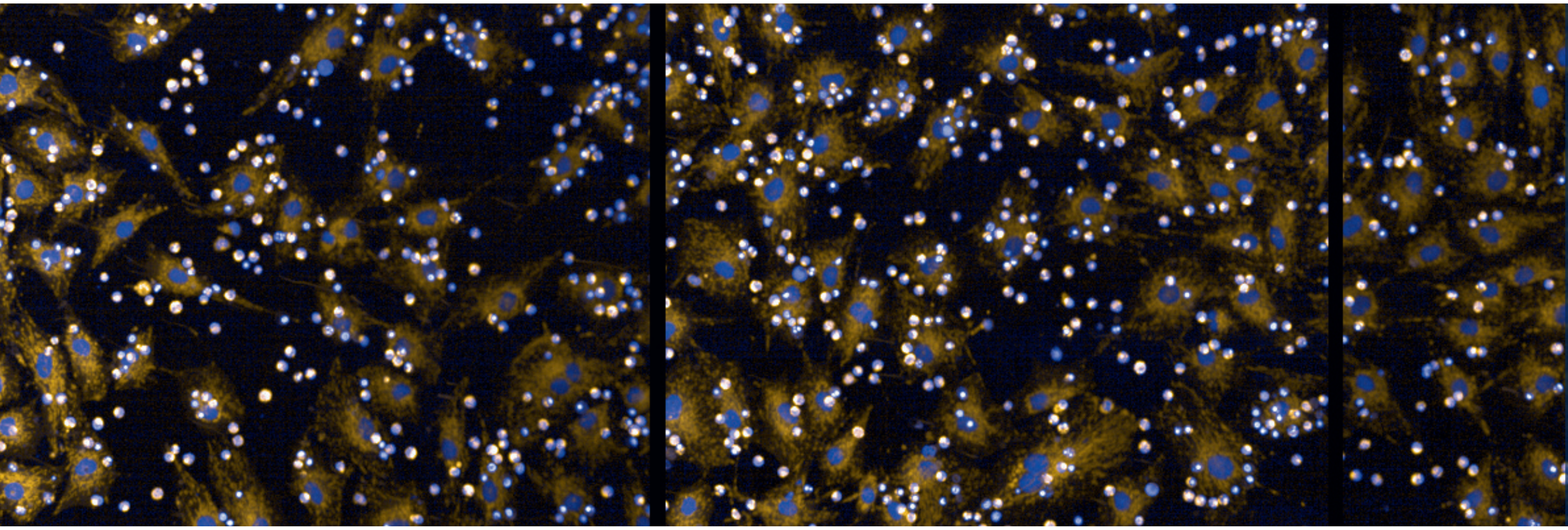

Ausgewählte Publikationen

ElHarouni D, Berker Y, Peterziel H, Gopisetty A, Turunen L, Kreth S, Stainczyk SA, Oehme I, Pietiäinen V, Jäger N, Witt O, Schlesner M, **Oppermann S.**
*iTReX: Interactive exploration of mono- and combination therapy dose response profiling data. **Pharmacol Res** 2022 Jan:175:105996. doi: 10.1016/j.phrs.2021.105996.*

Zeuner S, Vollmer J, Sigaud R, **Oppermann S**, Peterziel H, ElHarouni D, Oehme I, Witt O, Milde T, Ecker J.
*Combination drug screen identifies synergistic drug interaction of BCL-XL and class I histone deacetylase inhibitors in MYC-amplified medulloblastoma cells. **J Neurooncol**. 2024 Jan;166(1):99-112. doi: 10.1007/s11060-023-04526-w. Epub 2024 Jan 7. PMID: 38184819; PMCID: PMC10824805.*

H. Peterziel, N. Jamaladdin, ..., **Oppermann S**, Milde T, Witt O, Oehme I.
*Development of a functional patient-derived 3D multicellular platform for real-time personalized drug sensitivity profiling in the pediatric precision oncology program INFORM. **npj Precis Oncol.** 2022; doi: 10.1038/s41698-022-00335-y*

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venetoclax (VTX) in AML and identified rational combinatorial treatment strategies to overcome resistance. Sensitive (MOLM-13 S), BCL-2 G101V mutant (MOLM-13 G), and VTX-resistant (MOLM-13 VR) cells were analyzed using live-cell imaging (Hoechst, Annexin V, TMRE) and CellTiter-Glo assays. Western blotting revealed MCL-1 upregulation in resistant cells, indicating compensatory anti-apoptotic signaling. Dose-response curves showed ~1000-fold reduced sensitivity in resistant lines (IC50 ≈ 5 µM) versus sensitive MOLM-13 cells (IC50 ≈ 5 nM).

To identify potential therapeutic vulnerabilities, we performed a high-throughput screen of 135 compounds as mono- and combination treatments (± VTX at IC_{25}), using a library provided by the Structural Genomics Consortium (SGC). This library includes highly characterized and high-quality chemical probes from industry donations, now screened for repositioning opportunities. The initial screen identified eight compounds reducing cell viability as single agents across all tested AML models and some promising candidates that overcome VTX resistance in BCL-2 G101 V mutant AML cells. Validation studies extend to multiple concentrations, synergy matrices, additional AML lines, and primary blasts. Alongside protein-level resistance analyses, we integrate phenotypic imaging with molecular profiling to study drug-induced cellular and organelle changes. Future DRP experiments on primary AML samples using our oncology-focused drug library aim to advance clinical translation with FDA/EMA-approved drugs, novel targeted agents, and clinical phase II/III candidates.

Current developments and future directions:

We advance automated bioinformatic pipelines to quantitatively evaluate mono- and combination drug responses, integrating functional, molecular, and multi-omics data (ElHarouni et al., *Pharmacol. Res.*, 2022). This framework supports identification of cancer subtype–selective combinations and predictive biomarkers, providing a basis for precision medicine trials, including future basket studies. Concurrently, we enhance high-content

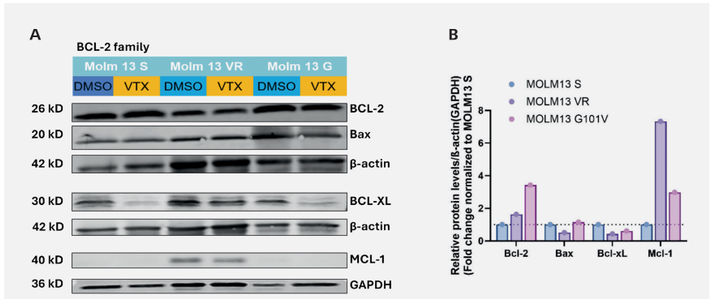

Figure 2. **Protein expression in MOLM13 S, VR, and G101V cells.** (**A**) Western blot of BCL-2 family proteins in MOLM13 S, VR, and G101V cells after DMSO or IC_{50} VTX treatment. The graph shows the fold change in relative protein levels, normalized to β-actin or GAPDH. (**B**) The graph shows the fold change in relative protein levels, normalized to ß-actin and DMSO of MOLM13 S (dotted line).

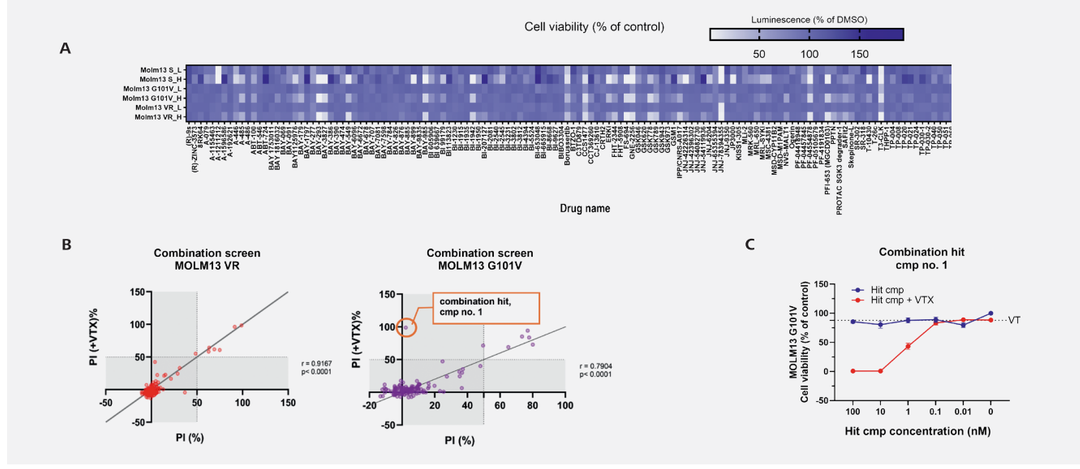

Figure 3: **TACTIC/DCP compounds or combinations screen.** (**A**) High-throughput drug screening heatmap showing cell viability (shown as normalized luminescence signal) of respective MOLM cell lines (S, VR, G) towards 135 compounds in 2 concentrations (L: 0.1 µM and H:10 µM). (**B**) 135 compounds ± VTX (IC25) in MOLM13 VR and G101V cells. (**C**) Cell viability of MOLM13 G101V cells after cmp no 1 as single agent (blue line) and in combination with VTX.

2D and 3D phenotypic image analysis using AI and deep learning. Multi-fluorescent live-cell assays capture single-cell phenotypes, enabling classification of drug responses, subpopulation-specific effects, and cell death mechanisms. Building on our deep transfer learning framework for patient-specific viability prediction prediction (Berker et al., *IEEE Trans. Med. Imaging*, 2022), these pipelines are expanded in collaboration with bioinformatics and AI partners. Together, these approaches create an integrated experimental and computational platform for functional precision oncology, facilitating mechanistic insights, discovery of novel drug combinations, and clinical translation of image- and AI-driven predictive assays. The group is fully affiliated with the Division of Clinical Pharmacy, FB 14, Institute of Pharmacology and Clinical Pharmacy, Goethe University Frankfurt.