A roadmap of therapeutic strategies for patients with multiple myeloma

Multiple myeloma is a rare and incurable cancer of plasma cells. To characterize this cancer, we developed an ex vivo drug screening method that combines imaging, deep learning and multiomics and applied it in an observational trial, uncovering new potential therapeutic strategies and underlying disease mechanisms.

This is a summary of:
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The mission

Despite the approval of chemotherapies, targeted therapies and immunotherapies for the treatment of multiple myeloma (MM), patients only have a 6-year median survival from diagnosis, and genetic profiling does little to help identify effective treatments. To help clinical treatment decision-making, we have developed an ex vivo image-based drug screening platform called pharmacoscopy (PCY)[1,2], which recently helped to identify potent treatments for patients with lymphoma and leukemia in a first-of-its-kind interventional trial[3,4]. The MM treatment landscape is rapidly evolving, and treatments regularly combine three or more drugs. Therefore, we set out to adapt PCY for MM, and aimed to use it to identify potent combinatorial treatment strategies, learn about drug sensitivity and resistance mechanisms, and understand if the approach is predictive of clinical response to therapies.

The discovery

We collaborated with the University Hospital Zurich to run a multi-year observational trial profiling over 100 bone marrow biopsy samples from patients with MM at all stages of the disease, in which we measured the response of each patient biopsy sample to a panel of combinatorial drugs using PCY in real-time (Fig. 1a). We tailored PCY to MM by developing a single-round multiplexed immunofluorescence assay, and classified all 729 million imaged patient cells using deep learning. Critically, we identified and genetically validated a morphological signature for malignant plasma cells, enabling accurate high-throughput drug response quantification. We integrated these measurements with matched proteomics data from purified plasma cells, serum cytokine profiling and clinical genetics. We also clinically annotated each sample, gathering details such as treatment history and treatment outcome after sampling. The resulting integration generated a comprehensive phenotypic, functional, and molecular view of the disease progression and heterogeneity of MM, which is accessible at https://myelomics.com.

Profiling drug responses ex vivo with PCY recapitulated key clinical observations, including the increased resistance of *TP53*-mutant samples to treatments that include proteasome inhibitors. Patients whose samples responded well to their next therapy ex vivo went on to stay on those treatments longer than patients with poor ex vivo responses, indicating the clinical predictive power of our PCY measurements (Fig. 1b). This was the case even for immunotherapies, where ex vivo and clinical therapy responses depend on a complex interplay between the patient's immune cells and cancer cells.

Integrative analyses implicated the negative mTOR-regulator DEPTOR in resistance to the proteasome inhibitor Bortezomib, and the expression of the transcriptional coactivator EYA3 in overcoming Bortezomib-induced genotoxic stress. We also observed that MMs that express MHC Class II respond well to the immunotherapy Elotuzumab ex vivo, coinciding with increased engagement of activated T cells with myeloma cells.

The cellular composition of the bone marrow biopsy samples markedly stratified the patient cohort into three phenogroups (PGs). These PGs corresponded to disease progression, inflammation and clonality, and strongly predicted ex vivo drug sensitivity. For example, PG2 samples, characterized by high T cell and monocyte infiltration and high inflammatory cytokine levels in the bone marrow of pre-treated patients, showed poor ex vivo and clinical responses to immunotherapy.

The implications

Our findings suggest new biomarker-based treatment strategies for MM, identify drug targets and resistance mechanisms, and reveal PCY to be a powerful tool to study MM and possibly tailor treatments to individual patients. The relative analytical simplicity of some of our findings, such as the PG classification, could be validated using routine clinical measurements or readily confirmed in ongoing clinical studies.

There are some limitations to the study. MM is such a heterogeneous disease that we should expand the study to more patients to untangle possible confounders and strengthen our findings. Furthermore, even with predefined analyses, observational studies have limited power in proving clinical impact. For this, well-controlled proof-of-concept and randomized interventional trials are necessary.

The next steps are to initiate interventional clinical studies in which PCY is used to tailor treatment to patients with late-stage MM. Further, we are working hard to expand PCY to bring benefit to patients with cancer who suffer from solid tumors. Early work in this direction from our group, available as a preprint article[5], uses PCY to identify approved drugs that can be repurposed for the treatment of primary brain tumors.

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EXPERT OPINION

"The ability to investigate cell–cell contacts via microscopy could be particularly important as immunotherapeutics become the most important drug class in MM. In addition, the integration of high content microscopy with emerging data-independent acquisition-based proteomics of primary patient samples is an advance. The ability to interrogate proteomic profiles as a function of drug response through an interactive data tool is highly useful." **Arun Wiita, University of California, San Francisco, San Francisco, CA USA.**

FIGURE

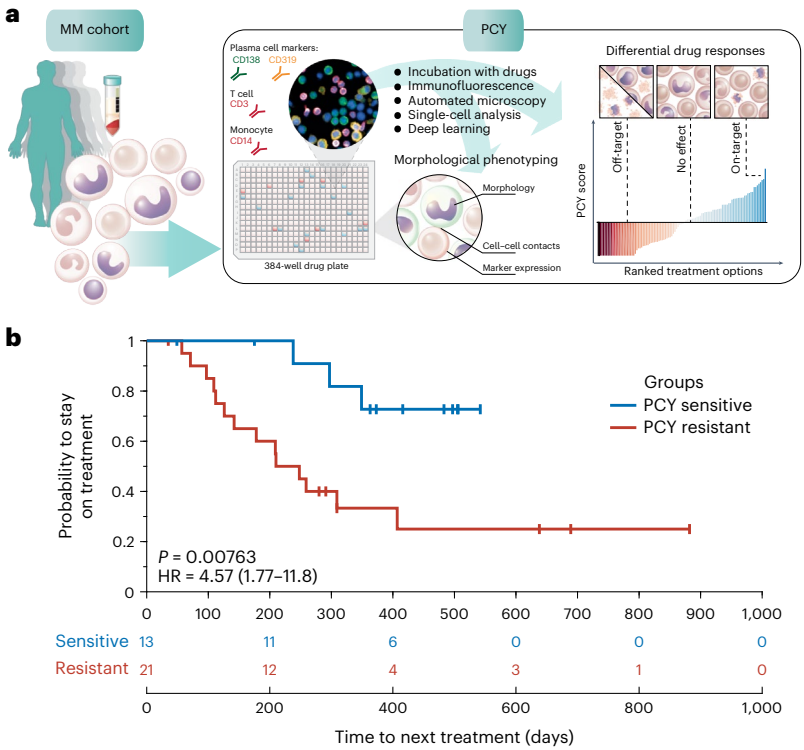

Fig. 1 | PCY workflow and clinical predictive power. a, Schematic detailing the use of PCY for the image-based ex vivo assessment of drug responses in biopsy samples from patients with MM. **b**, Kaplan–Meier curves of the probability to stay on treatment for 34 patients with MM, stratified by their ex vivo sensitivity to treatment measured prior to treatment initiation. Blue, above average responders; red, below average responders. *P*-value from log-rank (Mantel–Cox) test and hazard ratio (HR) including 95% confidence interval are reported. Ongoing responses are indicated as vertical tick marks. Number of patients at risk indicated in the table below.

BEHIND THE PAPER

This was the first clinical study I initiated in my new lab as Assistant Professor at the ETH Zurich back in 2017. The study was born out of mutual excitement with our clinical partners, and I was lucky enough to be awarded generous funding through an ERC Starting Grant. The first years were challenging, though. The sample and clinical heterogeneity appeared insurmountable, and the COVID-19 pandemic didn't help either. It wasn't until we figured out the morphological signature of myeloma cells that our results started to make clinical sense. Things rapidly fell into place from then on, although it still took a few years to collect the samples and essential clinical outcome data and perform follow-up experiments. Thanks to the entire team, particularly the first authors, for their persistence! **B.S.**

REFERENCES

1. Severin, Y. et al. Multiplexed high-throughput immune cell imaging reveals molecular health-associated phenotypes. *Sci. Adv.* **8**, eabn5631 (2022). **In this previous study from our group, we used PCY and deep learning to study immune cell morphology.**
2. Shilts, J. et al. A physical wiring diagram for the human immune system. *Nature* **608**, 397–404 (2022). **This study used protein–protein interaction mapping and PCY to uncover regulators of immune cell interactions.**
3. Snijder, B. et al. Image-based ex-vivo drug screening for patients with aggressive haematological malignancies: interim results from a single-arm, open-label, pilot study. *Lancet Haematol.* **4**, e595–e606 (2017). **This study provided the first evidence that PCY identifies effective treatments for patients with blood cancer.**
4. Kornauth, C. et al. Functional precision medicine provides clinical benefit in advanced aggressive hematologic cancers and identifies exceptional responders. *Cancer Discov.* **12**, 372–387 (2022). **The final analysis of this clinical study confirmed PCY-driven benefits for the treatment of blood cancers.**
5. Lee, S. et al., Targeting tumour-intrinsic neural vulnerabilities of glioblastoma. Preprint at *bioRxiv* https://doi.org/10.1101/2022.10.07.511321 (2022). **This preprint study using PCY on brain tumors revealed drug-repurposing candidates and associated mechanisms.**

FROM THE EDITOR

"This work by Snijder and colleagues stood out as an exciting strategy for precision cancer medicine, incorporating patient-specific image-based assessment of ex vivo sensitivity to a large panel of drugs and combinations, as well as multi-omics profiling. The authors have also made the data available in an online portal, as a valuable resource for the research community." **Editorial Team, *Nature Cancer*.**