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Competing interests

The authors declare no competing interests.

ONCOLOGY

Single-cell RNA sequencing: one step closer to the clinic

Single-cell RNA-sequencing can help in the prediction of drug resistance in patients with multiple myeloma.

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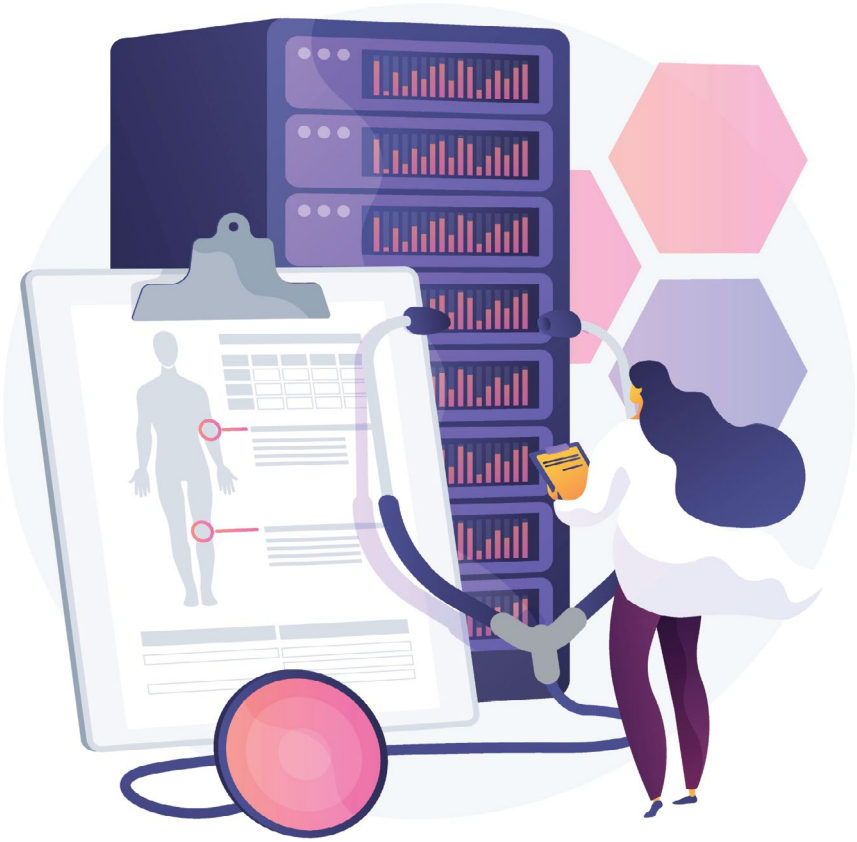
Multiple myeloma (MM), the second-most-common hematological malignancy in the USA, comprises an immunoglobulin-secreting plasma-cell tumor that resides in the bone marrow and can lead to permanent organ damage and death[1]. Despite substantial contributions by a highly active and devoted research community, MM remains a deadly disease, partly due to its marked genetic heterogeneity that complicates the discovery of universal biomarkers and the development of 'one-size-fits-all' therapy[2]. Although considerable advances in MM therapeutics have improved patient outcomes in recent years[3–5], a major clinical hurdle in curing this deadly and debilitating disease has been that most patients with MM eventually develop drug resistance and relapse[6,7]. It is therefore imperative to develop methods that can be used to predict resistance to treatment and alternative therapeutic options that prevent or circumvent the development of resistance. Now, in *Nature Medicine*, Cohen et al. report that single-cell RNA sequencing can be applied to patients' samples in the context of a clinical trial to help in the prediction of resistance and to guide therapeutic development to resensitize tumors to drugs[8].



Credit: Andrei Krauchuk / Alamy Stock Vector

In the study, the authors used single-cell RNA sequencing on bone-marrow samples from patients with primary refractory MM (PRMM) enrolled in the KYDAR trial (a single-arm prospective trial of daratumumab, carfilzomib, lenalidomide and dexamethasone (DARA-KRD)). Specifically, they performed MARS-seq (massively parallel single-cell RNA sequencing) on samples from a cohort of 41 patients with PRMM who either did not respond within the first 6 months of therapy or progressed early during the first 18 months of first-line treatment. Serial samples were obtained from some of these patients during treatment (cycles 4 and 10).

Comparing gene expression in tumor cells from patients with PRMM versus that in cells from healthy people, the authors identified an expression signature (module 1) that was upregulated in a subset of patients with PRMM. That module comprises genes encoding proteins related to

endoplasmic reticulum stress, the unfolded protein response, and the proteasome pathway, all of which are pathways relevant to resistance to first-line proteasome inhibitors.

Notably, the authors found that this transcriptional signature was an independent risk factor of both progression (progression-free survival) and death (overall survival) in the KYDAR trial and in an independent sample from the CoMMpass study, a cohort of ~1,000 patients with newly diagnosed MM for whom tumor DNA and bulk RNA-sequencing data are available[9]. These findings suggest that module 1 captures a resistance risk that is distinct from that captured by standard clinical risk-stratification systems. In fact, there is substantial overlap between module 1 and a DARA-KRD-resistance signature (generated by comparison of patients with PRMM who responded to DARA-KRD versus those who did not), which suggests that this resistance signature may be active and relevant throughout the course of several lines of treatment.

Next, to explore if the resistance signature could be used in clinics, in a prospective manner, the authors developed a model that can be applied to patients at baseline to predict their response to DARA-KRD. Although this model would be relevant for the ~30% of patients who already harbor resistant clones at baseline, it may also be relevant for the ~40% of patients that have small malignant clones that are undetectable or non-existent at baseline but drive progression through treatment selection and evolution. For such cases, by dynamically monitoring resistance in serial samples from the KYDAR trial, this approach identified resistant clones that appeared during treatment. This result suggests that dynamic monitoring of resistance while a patient is being treated may allow the early detection of an upcoming relapse, driven by small resistant clones.

While the use of gene-expression profiling for risk-stratification purposes has been previously applied to MM[10], the importance and novelty of this study lie in the concurrent profiling of tumor clonotypes with rearrangement of variable-diversity-joining regions (which serve as each tumor's unique identity tag[11,12]) and transcriptomes. In contrast to the profiling of tumors in bulk, single-cell sequencing approaches can differentiate among clones within the same tumor, which may or may not diverge substantially in their drug-resistance capacity. Therefore, this approach allows predictive scoring of individual clones in every tumor and thus may reveal smaller clones, some of which are predicted to be resistant, even though the majority of tumor cells may be sensitive to therapy. Such cases would presumably evolve under treatment and may need a different regimen to achieve durable remissions. If assessed in bulk, this signal would be diluted across all clones, which would perhaps make it undetectable, depending on the resistant clone's size. Furthermore, this approach also enables the detection of small resistant clones that appear while the patient is being treated. This is particularly interesting, as it underscores the utility of this method for both profiling patients before treatment initiation and dynamically assessing responses while they are being treated.

In the end, will this study change clinical practice? And if not, what is required for change? At present, single-cell RNA sequencing is still too expensive to be applied clinically as part of standard-of-care treatment, and using it to dynamically assess response in patients with MM would require serial bone-marrow biopsies, which are too invasive to be allowed. Furthermore, the resistance signature discovered in this study is unlikely to be relevant for every regimen and every patient. For example, although this signature seems to be an important predictor of resistance to proteasome inhibitors, it was present in only ~5% of newly diagnosed cases in the CoMMpass cohort. Other alterations (e.g., mutations in *CRBN*, which encodes cereblon) may be important for resistance to lenalidomide or other drugs[13]. Although this study is not expected to change clinical practice just yet, it does offer a blueprint for the development of a drug-resistance atlas that may indeed change clinical practice in the future. For this vision to become a reality, more whole-genome sequencing, single-cell RNA sequencing and/or sequencing of variable-diversity-joining regions, as well as proteomics, will need to be performed on patients being treated with different regimens. Larger longitudinal cohorts are needed in order to design comprehensive resistance tests that may need to span DNA, RNA and protein levels. By the time this vision is achieved, clinical single-cell sequencing may be feasible. ❐

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Competing interests

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