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Opinion

Targeting minimal residual disease: a path to cure?

Marlise R. Luskin[1], Mark A. Murakami[1], Scott R. Manalis[2-4*], David M. Weinstock[1,5*]

Affiliations: [1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, 02215, USA, [2]Koch Institute for Integrative Cancer Research, [3]Department of Biological Engineering and [4]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, and [5]Broad Institute of MIT and Harvard University, Cambridge, Massachusetts, 02142, USA.

*Corresponding authors: srm@mit.edu (S. R. M.) and davidm_weinstock@dfci.harvard.edu (D. M. W.)

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Preface

Therapeutics that block kinases, transcriptional modifiers, immune checkpoints and other biological vulnerabilities are transforming cancer treatment. As a result, many patients achieve dramatic responses, including complete radiographic or pathologic remissions, yet retain minimal residual disease (MRD) that results in relapse. New functional approaches can characterize clonal heterogeneity and predict therapeutic sensitivity of MRD at a single-cell level. Preliminary evidence suggests that iterative detection, profiling and targeting of MRD could meaningfully improve outcomes, and maybe even lead to cure.

Introduction

Complete remission (CR) in patients with cancer is traditionally defined as the absence of visible tumour using sensitive radiologic imaging (e.g. PET/CT or MRI scan) and, in some cases, histologic examination of tissue. Conventional chemotherapy regimens induce CR in the majority of patients with acute leukaemia or aggressive lymphoma.[1-4] In contrast, chemotherapy rarely induces CR in patients with metastatic carcinomas and sarcomas, multiple myeloma or chronic leukaemias.[5-10] The introduction of effective targeted therapies has changed the response paradigm for some patients, such as those with chronic myeloid leukaemia (CML), *EGFR*-mutated or *ALK*-rearranged lung adenocarcinoma, *KIT*-mutated gastrointestinal stromal tumours, and *BRAF*-mutated melanoma. Most patients with these diseases now achieve objective responses and in some cases even CR.[11-16] A panoply of therapeutics that target other kinases, transcriptional modifiers, immune checkpoints and other cancer vulnerabilities are currently under pre-clinical and clinical investigation. Used as monotherapies and in combinations, these agents are likely to usher in a new era, in which patients with advanced hematologic and solid cancers achieve CR in both the front-line and salvage settings.

CR, whether achieved by chemotherapy, targeted therapy, radiation, surgery, or a combination, typically requires >99% (i.e., >2-3 Log_{10}) reduction in tumour burden.[17, 18] In a hypothetical patient with 5 metastatic lesions averaging 2 cm^3 each, this would equate to a reduction from approximately 10^{10} tumour cells to $<10^8$ tumour cells (**Figure 1**). These remaining tumour cells have traditionally been called minimal residual disease (MRD). Not all MRD cells may be capable of contributing to a clinical relapse, so the term MRD is somewhat nonspecific. Although a full discussion is beyond the scope of this Perspective, we introduce a potentially useful nomenclature to distinguish different types of residual matter in **Box 1**.

For the sake of simplicity, we will define MRD as: malignant cells that remain in a patient who achieves CR and share phenotypic similarity (e.g. histologic appearance, lineage markers) and genetic heritage (e.g. truncal mutations and chromosomal rearrangements) with the original tumour cells. Residual cells that harbour somatic alterations and/or phenotypic alterations but are not fully malignant, such as those causing a dysplastic “field-effect”, are not included in this definition of MRD.

[H1] Rationale for targeting MRD

There are multiple conceptual advantages to treating patients with MRD-only disease rather than waiting for clinical relapse to initiate further therapy. First, the number of cancer cells is likely to be positively correlated with clonal complexity, and thus the likelihood of subclonal resistance to one or more therapeutics (**Figure 1**).[19] Second, minimal numbers of malignant cells may be less effective at remodelling microenvironments, reprogramming infiltrating hematopoietic cells and inducing chemoprotective niches.[20] As a result, certain drugs may have greater efficacy against MRD than against the same cancer at the time of clinical relapse. Third, the ability of patients to tolerate drugs with significant side effects may be better when only MRD is present compared to the time of fulminant relapse. Fourth, if cure requires the eradication of all tumour cells capable of driving relapse, there are likely fewer at the time of MRD. "Cancer stem cells" are less sensitive than more differentiated tumour cells to many drugs, but they are not completely resistant. Thus, it follows that "cancer stem cells" are enriched as a fraction of malignant cells within MRD but their total number is likely reduced in MRD compared to the time of frank relapse[21].

<u>[H3] Evidence that treating MRD can increase the rates of cure.</u> The strongest evidence that treatment of MRD can prevent relapse, which is a measurable surrogate for cure, comes from the experience using adjuvant (and to some extent neo-adjuvant) therapy for epithelial tumours and sarcomas, which is fundamentally intended to eradicate MRD outside the surgical resection. Studies across multiple cancer types have confirmed that more patients achieve long-term disease-free survival with the combination of surgery and adjuvant (or neoadjuvant) therapy than with surgery alone.[22-28]

Similarly, for hematologic cancers such as acute leukaemias or aggressive lymphomas, a single cycle of intensive chemotherapy can induce CR, but virtually no patients are cured without additional therapy to eradicate MRD.[29] In patients with acute leukaemia, flow cytometry of blood or bone marrow can detect aberrant immunophenotypic populations consistent with MRD at frequencies of ≤0.01% of cells.[17, 30-34] MRD detection with similar sensitivity is achievable by PCR or next-generation sequencing for gene fusions, patient-specific mutations, or clonal rearrangements of immunoglobulin (in B cells) or T-cell receptor (in T cells) genes. [17, 30, 33, 34] A “positive” test for MRD is not an absolute indicator of relapse, even upon completion of therapy, as the test may detect cells (or nucleic acids derived from those cells) that lack the ability to proliferate into a relapse [Box 1]. Nonetheless, a “positive” test for MRD in these patients, regardless of method, has repeatedly been associated with inferior prognosis.[33, 35-46]

[H3] Chemotherapy intensification based on MRD levels. Perhaps the most successful application of MRD-directed therapy is in children with acute lymphoblastic leukaemia (ALL), where tailoring consolidation therapy (*i.e.,* treatment after achieving a CR) based on MRD load is standard practice.[47] The presence or absence of MRD above defined thresholds directs relatively crude deintensification (*i.e.,* lower doses) or intensification of therapy (*i.e.,* higher doses with or without allogeneic stem cell transplantation); it does not guide the selection of targeted therapeutics to exploit the biological vulnerabilities of the MRD (**Figure 2**). Increasing levels of MRD after completion of therapy can inform clinicians about impending relapse. This may allow more time to plan salvage therapy but again does not guide the selection of targeted approaches to eradicate resistant clones.

[H3] Extended targeting of MRD: the CML experience. We propose that the next frontier for improving outcomes among patients who achieve CR is the precise targeting of MRD through genetic, transcriptional, functional and other predictive biomarkers. The treatment of CML and ALL carrying the BCR–ABL fusion kinase[48, 49] already incorporate genetic testing of MRD to guide therapeutic selection. Patients with inadequate initial responses to tyrosine kinase inhibitors (TKIs) or an increase in the amount of MRD following an initial response are commonly tested for the presence of BCR–ABL kinase domain mutations that confer TKI resistance. This testing can inform the rational selection of second or third-line therapy. Patients with CML who achieve optimal responses to first- or second-line TKI therapy (defined as sustained, deep remissions of greater than 4-5 $\log_{10}$ reductions in *BCR–ABL* transcript using the International Scale) may be candidates for drug cessation after multiple years of TKI therapy.[50] In recent trials, approximately one-half of patients who discontinue therapy under those circumstances will remain in prolonged molecular remissions.[50-55] The last reported median follow-up from the Stop Imatinib (STIM1) trial of imatinib discontinuation reached 77 months (range 9-95 months), with similar follow-up for those alive and without molecular recurrence (80 months, range 55-93 months).[55] Follow-up for the STOP second generation (2G)-TKI study of dasatinib and nilotinib discontinuation has a median of 47 months (range 12-65 months).[54]

The experience with CML provides two potential insights relevant to curing cancer based on iterative MRD testing. First, relatively long-term treatment with continuous therapeutic pressure may be necessary to completely eradicate the malignant cells. The reasons for this remain poorly understood but one possibility is that "CML stem cells" are primarily quiescent and only intermittently enter cell cycle.[56, 57] While quiescent, they fail to undergo oncogene withdrawal-induced apoptosis, although this apoptosis is readily induced by TKIs in more differentiated progeny.[58] Complete eradication of the quiescent population may require that adequate inhibition of BCR–ABL kinase activity continues for long enough to allow each CML stem cell the chance to enter cell cycle and thereby become susceptible to oncogene withdrawal-induced apoptosis. Populations of cells with "stem cell-like" properties exist in many types of cancer[59, 60] suggesting that the requirement for extended therapy may be common.

The same concept of long-term therapeutic pressure is also established for ALL maintenance therapy with the thiopurines 6-mercaptopurine (6-MP) or 6-thioguanine (6-TG), which are given in combination with other "low-dose" chemotherapy agents for 2 to 3 years.[61, 62] Resistant clones that emerge late in this maintenance phase frequently harbour mutations in *NT5C2* that directly confer resistance to 6-MP and 6-TG.[63, 64] This mechanism of tumour cell-autonomous resistance suggests that thiopurines improve cure rates by directly killing the last leukaemia cells capable of driving relapse (i.e. the MRD), rather than acting through non-tumour cell autonomous mechanisms.

The second insight from the CML experience is that patients who inadequately respond to or relapse during first-line TKI treatment may still be curable with a second-line TKI that retains efficacy.[53, 54] Thus, the presence of resistance mutations within BCR–ABL does not eliminate the potential for CML to be cured, as long as therapeutics are available that overcome the resistance and can adequately inhibit BCR–ABL kinase activity for a long enough period of time.

This paradigm, in which a cancer retains equivalent "curability" in the first- and second-line settings may be more broadly applicable to other cancers treated with targeted therapies. However, it may not apply to cancers treated with clastogenic chemotherapies, as these agents induce genetic diversity and thus may promote the development of subclones that intrinsically lack responsiveness to other therapeutics (e.g. by genetic loss of genes encoding apoptosis effectors[65]).

[H1] Approaches to characterize MRD

[H3] Biological and functional challenges. An optimal assay performed at the time of CR would not only predict therapeutic response but would also identify relevant heterogeneity that is present within the patient's MRD. It would utilize very small amounts of input material, generate easily interpretable data within a short period, and require relatively little operator skill. Such an assay could be iteratively applied to assess MRD and guide therapeutic selection (as in **Figure 1**). This would be a transformative clinical advance for patients with persistent MRD if selective targeting of that MRD either forestalled or completely obviated clinical relapse.

A major concern with characterizing MRD is that sampling from a single site could result in misrepresentation of the true heterogeneity *in situ*. To some extent, heterogeneity within MRD can be addressed by iterative sampling, testing and therapeutic targeting. When the MRD is then reassessed, subclones (or cellular contents from them) that were not effectively targeted based on the previous assessment should be enriched within the sampled population.

There are theoretical challenges to bioassay-based targeting of solid tumour MRD that suggest the primary focus for this strategy should be on hematologic cancers. For example, independent metastases from the same carcinoma can undergo divergent evolution that leads to differential therapeutic response, and even primary solid tumours can have extensive geographic heterogeneity.[66-68] There is a widely held belief that hematologic cancers may be less geographically heterogeneous than carcinomas but this has not been systematically proven. The larger advantage for sampling MRD in patients with leukaemias and some lymphomas is that MRD cells can be isolated from the bone marrow. In contrast, the sites of MRD within a patient who underwent resection of his or her primary cancer are almost always unknown. Thus, two issues preclude MRD sampling for many patients with solid tumours: 1) there is inadequate signal-to-noise ratio for detection of MRD using currently available imaging approaches and 2) biopsy of sites like liver, bone, spleen and lung requires invasive sampling. The latter would have to be justified by improvements in outcome that come from therapeutic decisions based on analysis of the sampled material.

Assays that utilize circulating tumour cells (CTCs) and cell-free nucleic acids may be feasible for testing MRD in the future but are currently limited by the quantity of malignant material recovered by phlebotomy.[69-71] A more invasive and costly approach like pheresis could sample litres of blood from a patient to access larger numbers of circulating malignant cells or other material.[72] Like invasive biopsies, an approach like pheresis may be merited if therapeutic selection based on a predictive assay performed using that material significantly improved patient outcome. Alternatively, agents that interrupt tumour cell interactions with the microenvironment, such as those targeting CXCR4 or E-selectin, could be used to transiently increase the number of CTCs in a patient with MRD; importantly, concerns have been raised that this type of approach may increase metastasis.[73] Finally, one could imagine testing multiple different sites of MRD from a patient with micrometastases if technological advances allowed for identification of those sites (e.g. with new imaging strategies) and minimally invasive sampling (e.g. with microneedles).

[H3] The argument for functional assays. Recent advances in cell-free nucleic acid characterization have made it possible to make genotype-driven therapeutic selections in some patients, such as those with *EGFR*-mutated lung cancer.[74-78] In tumours where a targeted agent is available and associated with genotype-specific activity (i.e. the presence or absence of resistance mutations), sequencing of circulating tumour DNA could even be used to inform the selection of specific agents, as in CML. Unfortunately, nucleic acid-based biomarkers that predict sensitivity or resistance to cancer therapeutics are the exception rather than the rule.[79]

For the overwhelming majority of cytotoxic chemotherapies and many targeted agents, there are few if any genetic, transcriptional, proteomic, metabolomic or other biomarkers to predict the depth or duration of response. As a result, functional assays that directly measure phenotypic responses to single agents or therapeutic combinations are particularly attractive. We will primarily focus on functional assays, as they capture complex and multiparametric interactions to predict *in vivo* therapeutic efficacy. However, there are several non-functional assays that have the potential to define aspects of intratumoural biology and even link changes associated with drug exposure to clinical outcome. Among these, mass cytometry can quantify over 40 parameters, including protein levels, posttranslational modifications and proteolysis products, from millions of single cells within an individual sample[80]. Mass cytometry can reveal cell signaling programs as well as markers of intercellular communication (e.g. cytokines or growth factors) that reflect the surrounding environment. Single-cell RNA sequencing (scRNA-seq) is similarly capable of defining multiple aspects of cell state as well as genotype.[81] Microfluidic approaches have now made it possible to conduct scRNA-seq or other sequencing platforms on thousands of cells at low cost[82-84], revealing intratumoural heterogeneity in both malignant and non-malignant populations.[82, 85, 86] Yet, these vast insights into tumour biology have not thus far been translated into clinically relevant approaches for selecting therapeutics for an individual patient.[79]

[H3] The evolution of functional assays. Functional testing of therapeutic sensitivity of individual cancers initially followed similar paradigms that were overwhelmingly successful for determining antibiotic sensitivity in bacteria[87-89]. Tumour cells were plated in 2-dimensional (2-D) culture *ex vivo* in the presence of potentially-active therapeutics and some marker of either survival or proliferation was measured. In contrast to antibiotic sensitivity testing, bulk assays for cancer therapeutic sensitivity were generally not useful for several reasons. First, extended culture of tumour cells is difficult, so comparisons between untreated and drug-treated cells are largely a "race to death". Second, available assays required relatively robust laboratory capabilities within a short distance from the patient bedside, as extended transport further compromises tumour cell survival. Third, the prolonged culture necessary to readout survival or growth resulted in significant selection for cells with characteristics that favour *in vitro* survival. Finally, and importantly, these assays were primarily conducted in an era when cancer therapeutics were not very active against most solid tumours, so the chances of identifying a highly effective therapy (including combinations) was *a priori* low.

There are several strategies for overcoming issues related to selection in 2-D culture. Multiple groups have established either organoids from individual patient tumours or patient-derived tumour xenografts (PDXs). Both strategies may capture relevant interactions that mimic the *in situ* microenvironment but also require extended propagation that allows for clonal selection and a temporal gap between the biopsy and the readout for drug efficacy.[90-93]

An alternative approach is to either eliminate or reduce the need for *ex vivo* propagation by using a rapid readout of single cell treatment response. Several assays have been reported that can measure proteomic, transcriptional, functional or other readouts of drug response within cancer cells and could be tested on MRD. An inexhaustive sampling is included in **Table 1**.

One promising approach for assaying the effects of multiple drugs on a single tumour specimen *ex vivo* is dynamic BH3 profiling (DBP). This assay quantifies treatment-induced changes in net pro-apoptotic signalling within mitochondria.[94] In DBP, patient tumour cells are exposed to drugs for 16-24 hours *ex vivo*, permeabilized and exposed to proapoptotic, BH3-domain-containing peptides. The frequency of cells that undergo mitochondrial outer membrane permeabilization is measured to assess the extent to which tumour cells are "primed" for apoptosis. The difference between priming in the presence and absence of exposure to a therapy within the bulk tumour population is used to predict whether the therapy will be effective in the patient.[95] DBP has been used to accurately predict the response of CML to imatinib using bone marrow samples, the response of single cell suspensions from ovarian cancer biopsies to carboplatin, the response of ALL PDXs to MDM2 inhibition and in other settings.[94, 96, 97]

Although many drugs have yet to be tested using DBP, the assay is likely to be amenable to a wide range of targeted therapies that act through the intrinsic apoptotic pathway in tumour cells. Like 2-D culture, organoids, and PDXs, DBP queries the treatment response of tumour cells in bulk rather than individual cells, although advances in flow cytometry-based DBP may allow for its application to single cells.[98] Other approaches that utilize flow cytometry could also be applied to assay the effects of therapeutics when exposed to samples *ex vivo*.[99] Flow cytometry-based assays offer the added benefit that 10^5-10^6 cells can be easily analysed from a single sample, which allows for the interrogation of MRD present at frequencies less than 1-in-10,000 cells.

Together with colleagues at the Massachusetts Institute of Technology (MIT) and Dana-Farber Cancer Institute (DFCI), we have been testing whether a measurement device for single cells known as the suspended microchannel resonator (SMR) can be applied to assess functional drug response in single cancer cells (**Figure 3**). When an object denser than media passes through the SMR, the net increase in mass (i.e. the buoyant mass of the object) lowers the resonant frequency. To measure a cell's mass accumulation rate (MAR), an array of SMRs are microfluidically connected in series, with "delay" channels in between each cantilever. Passage through the delay channels provides the cell with time to gain or lose biomass before the next cantilever. After a cell exits a cantilever, other cells are free to enter it and be weighed. Over 100 cells per hour can pass through the array in a queue, each being measured with a precision near 0.01% of the cell's weight.[100, 101] MAR is a measurement of cell growth that does not require proliferation. Thus, like DBP, the MAR assay can rapidly assess drug response whether that drug is applied *in situ* or *ex vivo*.[102]

We have demonstrated that the MAR assay can define the drug sensitivity or resistance of glioblastoma and B-cell ALL cells to targeted agents.[102] For multiple myeloma, we have recently demonstrated that the MAR assay accurately and rapidly defines therapeutic susceptibility in human multiple myeloma cell lines. In a study of 6 patients who responded to therapy that included the proteasome inhibitor bortezomib and 3 who did not, MAR correctly predicted response with 100% accuracy.[103] Importantly, the MAR assay is nondestructive, which allows both sensitive and resistant cells to be interrogated by live-cell downstream assays such as scRNA-seq or DBP (**Figure 3B)**. Because these properties are measured for each single cell, clonal architectures based on therapeutic response can ultimately be established across each tumour sample by incorporating molecular and functional measurements from large numbers of cells. In settings of deep treatment response, pre-treatment and MRD samples can be compared to define the effects of therapy on clonal architecture. We envision these data could then be incorporated into mathematical models to design and optimize therapeutic approaches that address the heterogeneity within individual tumours that results in treatment failure.

There are multiple additional methods for measuring biophysical properties of single cells with high throughput that have not yet been fully exploited for assessing drug responses. For example, microscopy can quantify cell size and morphology[104] and several different microfluidic approaches have been used to interrogate mechanical properties of single cells[105-108]. Label-independent techniques that exploit differences in the size, shape, or rigidity of malignant versus non-malignant cells also exist but haven't yet been fully exploited for identifying MRD cells.

[H3] Tumour cell enrichment and assay sensitivity. For most platforms listed in **Table 1**, tumour cells must be pre-enriched before assaying drug response as the throughput is insufficient to capture an adequate number of malignant cells within a sample containing primarily non-malignant cells. Pre-enrichment typically involves targeting cell surface proteins using antibodies conjugated to magnetic beads or fluorescent molecules followed by either flow-associated cell sorting or positive selection with magnetic beads.[109-112]

Even with advances in tumour cell enrichment, there is a minimum threshold for involvement of MRD below which an assay is simply not feasible. For example, a recent study on MRD in multiple myeloma used next-generation flow cytometry on bone marrow to identify ~2 MRD cells per million.[113] Although identification of MRD at this frequency is now possible, sorting and then drug testing these cells is unlikely to be possible. However, testing frequencies of MRD between 1 in 10,000 to 1 in 1,000 cells should be feasible based on the ability to sequence individual cells present at even lower frequencies.[114] In a sample of 10^6-10^7 cells, this would result in hundreds to thousands of MRD cells that could be interrogated for drug response. To evaluate a panel of drugs with this number of total MRD cells, the platforms listed in Table 1 will require new and innovative approaches for cell handling. Sampling this many tumour cells from patients in CR after treatment of solid tumours may be particularly difficult, although it is likely to be feasible using carcinoma and sarcoma resections after neoadjuvant therapy.[115-117]

[H1] The future of MRD=guided therapy

It remains to be determined whether applying any functional assay to MRD samples can lead to the selection of treatments that extend the duration of CR over current empiric approaches. However, it seems intuitive that a greater appreciation of heterogeneity in the response of individual MRD cells to a therapy (including combinations) can inform therapeutic selection, provided therapies exist that are capable of overcoming the resistance. It also seems intuitive that patients will accept more invasive sampling, even multiple biopsies, at the time of MRD if that sampling has been proven to inform therapeutic selection and confer meaningful benefit.

As newer and more effective agents are introduced for patients with cancer, we can now envision preventing disease relapse through the rational targeting of MRD. To achieve that ambitious goal will require carefully designed trials in selected settings that apply novel approaches for sampling and testing live MRD cells. One can envision two paradigms for targeting MRD. In the first, patients would be treated until MRD is no longer detectable and then given drug-free holidays until MRD relapses (as in **Figure 1**). At that time, MRD would be assessed for therapeutic sensitivity and the appropriate treatment (re)-initiated. This is a form of "preemptive" therapy like the suppression of viral reactivations among immunocompromised patients[118]. In the second paradigm, treatment would continue either indefinitely (akin to secondary prophylaxis of infections) or with curative intent. The ability to "cure" a tumour will depend on several factors, most notably the efficacy and lack of cross-resistance among therapeutics for that disease.

Clinical settings ripe for initial trials include hematologic neoplasms in which patients commonly achieve CR to initial therapy but are prone to relapse from MRD after completion of therapy, such as acute and chronic leukaemias, lymphomas, and multiple myeloma. Multiple drugs have single-agent activity in these patients but few biomarkers are available to guide drug selection.

In a trial that tests functional assessment of MRD, patients could be randomized to either receive the treatment predicted to be optimal based on MRD testing or to receive the treating physician's choice of therapy. Upon treatment failure (either upfront failure or after a period of response), MRD cells would again be isolated from patients. The cells would be tested using the functional assay and the results would guide therapeutic selection (if randomized to that arm) or kept confidential (if randomized to physician's choice). This would continue iteratively until the patient was either cured or underwent a clinical relapse. The trial would be designed to assess the approach (i.e., informed testing of MRD) rather than the efficacy of any single drug.

Several issues in the design of such a trial remain. Surrogate (eradication of detectable MRD) and definitive (relapse free survival) endpoints will need to be carefully selected to assess for superiority of a rational MRD-targeted treatment approach. Factors such as the frequency of MRD testing, the threshold for therapeutic actionability, and issues of standardization and generalizability of implementation will need to be addressed, as will the availability of multiple potentially active therapeutics.

In sum, there is much work to be done, but to quote Theodore Roosevelt, "Far and away the best prize that life offers is the chance to work hard at work worth doing."[119]

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Marlise R. Luskin1, Mark A. Murakami1, Scott R. Manalis2-4*, David M. Weinstock1,5*

Author contributions

M. R. L, M. A. M., S. R. M. and D. M. W. researched data for the article, substantially contributed to discussion of content, wrote the article, and reviewed and edited the manuscript before submission.

Competing interests statement

The authors declare competing interests; see Web version for details.

References

1. Buchner, T. et al. Acute Myeloid Leukemia (AML): different treatment strategies versus a common standard arm--combined prospective analysis by the German AML Intergroup. *J Clin Oncol* **30**, 3604-10 (2012).
2. Terwilliger, T. & Abdul-Hay, M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood Cancer J* **7**, e577 (2017).
3. Armitage, J.O. The aggressive peripheral T-cell lymphomas: 2017. *Am J Hematol* **92**, 706-715 (2017).
4. Kahl, B. Chemotherapy combinations with monoclonal antibodies in non-Hodgkin's lymphoma. *Semin Hematol* **45**, 90-4 (2008).
5. Scagliotti, G.V. et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* **26**, 3543-51 (2008).
6. Souglakos, J. et al. FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) vs FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) as first-line treatment in metastatic colorectal cancer (MCC): a multicentre randomised phase III trial from the Hellenic Oncology Research Group (HORG). *Br J Cancer* **94**, 798-805 (2006).
7. Nabholtz, J.M. et al. Phase II study of docetaxel, doxorubicin, and cyclophosphamide as first-line chemotherapy for metastatic breast cancer. *J Clin Oncol* **19**, 314-21 (2001).
8. Chapman, P.B. et al. Phase III multicenter randomized trial of the Dartmouth regimen versus dacarbazine in patients with metastatic melanoma. *J Clin Oncol* **17**, 2745-51 (1999).
9. Casper, E.S. Gastrointestinal stromal tumors. *Curr Treat Options Oncol* **1**, 267-73 (2000).
10. Tannock, I.F. et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* **351**, 1502-12 (2004).
11. Maemondo, M. et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* **362**, 2380-8 (2010).
12. Soria, J.C. et al. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. *Lancet* **389**, 917-929 (2017).
13. Mazieres, J. et al. Crizotinib therapy for advanced lung adenocarcinoma and a ROS1 rearrangement: results from the EUROS1 cohort. *J Clin Oncol* **33**, 992-9 (2015).
14. Long, G.V. et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N Engl J Med* **371**, 1877-88 (2014).
15. Ascierto, P.A. et al. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. *Lancet Oncol* **17**, 1248-60 (2016).
16. Blanke, C.D. et al. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. *J Clin Oncol* **26**, 626-32 (2008).
17. Hourigan, C.S. & Karp, J.E. Minimal residual disease in acute myeloid leukaemia. *Nat Rev Clin Oncol* **10**, 460-71 (2013).
18. Adler, S. et al. Minimum lesion detectability as a measure of PET system performance. *EJNMMI Phys* **4**, 13 (2017).

19. Rodriguez-Brenes, I.A. & Wodarz, D. Preventing clonal evolutionary processes in cancer: Insights from mathematical models. *Proc Natl Acad Sci U S A* **112**, 8843-50 (2015).
20. McGranahan, N. & Swanton, C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. *Cell* **168**, 613-628 (2017).
21. Terwijn, M. et al. Leukemic stem cell frequency: a strong biomarker for clinical outcome in acute myeloid leukemia. *PLoS One* **9**, e107587 (2014).
22. Wolmark, N. et al. Postoperative adjuvant chemotherapy or BCG for colon cancer: results from NSABP protocol C-01. *J Natl Cancer Inst* **80**, 30-6 (1988).
23. Wolmark, N. et al. The benefit of leucovorin-modulated fluorouracil as postoperative adjuvant therapy for primary colon cancer: results from National Surgical Adjuvant Breast and Bowel Project protocol C-03. *J Clin Oncol* **11**, 1879-87 (1993).
24. Andre, T. et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. *J Clin Oncol* **27**, 3109-16 (2009).
25. Early Breast Cancer Trialists' Collaborative, G. et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet* **379**, 432-44 (2012).
26. Pervaiz, N. et al. A systematic meta-analysis of randomized controlled trials of adjuvant chemotherapy for localized resectable soft-tissue sarcoma. *Cancer* **113**, 573-81 (2008).
27. Yu, Z. et al. Adjuvant endocrine monotherapy for postmenopausal early breast cancer patients with hormone-receptor positive: a systemic review and network meta-analysis. *Breast Cancer* (2017).
28. Winton, T. et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. *N Engl J Med* **352**, 2589-97 (2005).
29. Frei, E., 3rd et al. The effectiveness of combinations of antileukemic agents in inducing and maintaining remission in children with acute leukemia. *Blood* **26**, 642-56 (1965).
30. Kayser, S., Schlenk, R.F., Grimwade, D., Yosuico, V.E. & Walter, R.B. Minimal residual disease-directed therapy in acute myeloid leukemia. *Blood* **125**, 2331-2335 (2015).
31. Wood, B.L. Flow cytometric monitoring of residual disease in acute leukemia. *Methods Mol Biol* **999**, 123-36 (2013).
32. Theunissen, P. et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. *Blood* **129**, 347-357 (2017).
33. Ossenkoppele, G. & Schuurhuis, G.J. MRD in AML: does it already guide therapy decision-making? *Hematology Am Soc Hematol Educ Program* **2016**, 356-365 (2016).
34. Campana, D. Minimal residual disease in acute lymphoblastic leukemia. *Hematology Am Soc Hematol Educ Program* **2010**, 7-12 (2010).
35. Bruggemann, M. et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. *Blood* **107**, 1116-23 (2006).
36. Bruggemann, M., Raff, T. & Kneba, M. Has MRD monitoring superseded other prognostic factors in adult ALL? *Blood* **120**, 4470-81 (2012).
37. Grimwade, D. et al. Prospective minimal residual disease monitoring to predict relapse of acute promyelocytic leukemia and to direct pre-emptive arsenic trioxide therapy. *J Clin Oncol* **27**, 3650-8 (2009).

38. Yin, J.A. et al. Minimal residual disease monitoring by quantitative RT-PCR in core binding factor AML allows risk stratification and predicts relapse: results of the United Kingdom MRC AML-15 trial. *Blood* **120**, 2826-35 (2012).
39. Beldjord, K. et al. Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. *Blood* **123**, 3739-49 (2014).
40. Borowitz, M.J. et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. *Blood* **111**, 5477-85 (2008).
41. Chen, X. et al. Relation of Clinical Response and Minimal Residual Disease and Their Prognostic Impact on Outcome in Acute Myeloid Leukemia. *J Clin Oncol* (2015).
42. Freeman, S.D. et al. Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. *J Clin Oncol* **31**, 4123-31 (2013).
43. Gokbuget, N. et al. Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. *Blood* **120**, 1868-76 (2012).
44. Terwijn, M. et al. High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from the HOVON/SAKK AML 42A study. *J Clin Oncol* **31**, 3889-97 (2013).
45. Cave, H. et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer--Childhood Leukemia Cooperative Group. *N Engl J Med* **339**, 591-8 (1998).
46. Vidriales, M.B. et al. Minimal residual disease in adolescent (older than 14 years) and adult acute lymphoblastic leukemias: early immunophenotypic evaluation has high clinical value. *Blood* **101**, 4695-700 (2003).
47. Pieters, R. et al. Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group. *J Clin Oncol* **34**, 2591-601 (2016).
48. Ravandi, F. et al. Detection of MRD may predict the outcome of patients with Philadelphia chromosome-positive ALL treated with tyrosine kinase inhibitors plus chemotherapy. *Blood* **122**, 1214-21 (2013).
49. Cortes, J. & Kantarjian, H. How I treat newly diagnosed chronic phase CML. *Blood* **120**, 1390-7 (2012).
50. Hughes, T.P. & Ross, D.M. Moving treatment-free remission into mainstream clinical practice in CML. *Blood* **128**, 17-23 (2016).
51. Ross, D.M. et al. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study. *Blood* **122**, 515-22 (2013).
52. Rousselot, P. et al. Loss of major molecular response as a trigger for restarting tyrosine kinase inhibitor therapy in patients with chronic-phase chronic myelogenous leukemia who have stopped imatinib after durable undetectable disease. *J Clin Oncol* **32**, 424-30 (2014).
53. Imagawa, J. et al. Discontinuation of dasatinib in patients with chronic myeloid leukaemia who have maintained deep molecular response for longer than 1 year (DADI trial): a multicentre phase 2 trial. *Lancet Haematol* **2**, e528-35 (2015).

54. Rea, D. et al. Discontinuation of dasatinib or nilotinib in chronic myeloid leukemia: interim analysis of the STOP 2G-TKI study. *Blood* **129**, 846-854 (2017).
55. Etienne, G. et al. Long-Term Follow-Up of the French Stop Imatinib (STIM1) Study in Patients With Chronic Myeloid Leukemia. *J Clin Oncol* **35**, 298-305 (2017).
56. Michor, F. Quantitative approaches to analyzing imatinib-treated chronic myeloid leukemia. *Trends Pharmacol Sci* **28**, 197-9 (2007).
57. Michor, F. et al. Dynamics of chronic myeloid leukaemia. *Nature* **435**, 1267-70 (2005).
58. Jorgensen, H.G., Allan, E.K., Jordanides, N.E., Mountford, J.C. & Holyoake, T.L. Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+ CML cells. *Blood* **109**, 4016-9 (2007).
59. Tirosh, I. et al. Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma. *Nature* **539**, 309-313 (2016).
60. Valent, P. et al. Cancer stem cell definitions and terminology: the devil is in the details. *Nat Rev Cancer* **12**, 767-75 (2012).
61. Lonsdale, D. et al. Interrupted vs. continued maintenance therapy in childhood acute leukemia. *Cancer* **36**, 341-52 (1975).
62. Rivera, G.K., Pinkel, D., Simone, J.V., Hancock, M.L. & Crist, W.M. Treatment of acute lymphoblastic leukemia. 30 years' experience at St. Jude Children's Research Hospital. *N Engl J Med* **329**, 1289-95 (1993).
63. Tzoneva, G. et al. Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL. *Nat Med* **19**, 368-71 (2013).
64. Meyer, J.A. et al. Relapse-specific mutations in NT5C2 in childhood acute lymphoblastic leukemia. *Nat Genet* **45**, 290-4 (2013).
65. Meijerink, J.P. et al. Hematopoietic malignancies demonstrate loss-of-function mutations of BAX. *Blood* **91**, 2991-7 (1998).
66. Juric, D. et al. Convergent loss of PTEN leads to clinical resistance to a PI(3)Kalpha inhibitor. *Nature* **518**, 240-4 (2015).
67. Jamal-Hanjani, M. et al. Tracking the Evolution of Non-Small-Cell Lung Cancer. *N Engl J Med* **376**, 2109-2121 (2017).
68. Gerlinger, M. et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med* **366**, 883-892 (2012).
69. Micalizzi, D.S., Maheswaran, S. & Haber, D.A. A conduit to metastasis: circulating tumor cell biology. *Genes Dev* **31**, 1827-1840 (2017).
70. Karabacak, N.M. et al. Microfluidic, marker-free isolation of circulating tumor cells from blood samples. *Nat Protoc* **9**, 694-710 (2014).
71. de Bono, J.S. et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin Cancer Res* **14**, 6302-9 (2008).
72. Fischer, J.C. et al. Diagnostic leukapheresis enables reliable detection of circulating tumor cells of nonmetastatic cancer patients. *Proc Natl Acad Sci U S A* **110**, 16580-5 (2013).
73. Martin, O.A., Anderson, R.L., Narayan, K. & MacManus, M.P. Does the mobilization of circulating tumour cells during cancer therapy cause metastasis? *Nat Rev Clin Oncol* **14**, 32-44 (2017).
74. Wan, J.C. et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat Rev Cancer* **17**, 223-238 (2017).

75. Douillard, J.Y. et al. Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. *J Thorac Oncol* **9**, 1345-53 (2014).
76. Reck, M. et al. ctDNA Determination of EGFR Mutation Status in European and Japanese Patients with Advanced NSCLC: The ASSESS Study. *J Thorac Oncol* **11**, 1682-9 (2016).
77. Sacher, A.G. et al. Prospective Validation of Rapid Plasma Genotyping for the Detection of EGFR and KRAS Mutations in Advanced Lung Cancer. *JAMA Oncol* **2**, 1014-22 (2016).
78. Remon, J. et al. Osimertinib benefit in EGFR-mutant NSCLC patients with T790M-mutation detected by circulating tumour DNA. *Ann Oncol* **28**, 784-790 (2017).
79. Letai, A. Functional precision cancer medicine-moving beyond pure genomics. *Nat Med* **23**, 1028-1035 (2017).
80. Spitzer, M.H. & Nolan, G.P. Mass Cytometry: Single Cells, Many Features. *Cell* **165**, 780-91 (2016).
81. Shalek, A.K. & Benson, M. Single-cell analyses to tailor treatments. *Sci Transl Med* **9** (2017).
82. Gierahn, T.M. et al. Seq-Well: portable, low-cost RNA sequencing of single cells at high throughput. *Nat Methods* **14**, 395-398 (2017).
83. Klein, A.M. et al. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell* **161**, 1187-201 (2015).
84. Macosko, E.Z. et al. Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. *Cell* **161**, 1202-14 (2015).
85. Prakadan, S.M., Shalek, A.K. & Weitz, D.A. Scaling by shrinking: empowering single-cell 'omics' with microfluidic devices. *Nat Rev Genet* **18**, 345-361 (2017).
86. Tirosh, I. et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* **352**, 189-96 (2016).
87. Friedman, A.A., Letai, A., Fisher, D.E. & Flaherty, K.T. Precision medicine for cancer with next-generation functional diagnostics. *Nat Rev Cancer* **15**, 747-56 (2015).
88. Grendys, E.C. et al. Overview of a chemoresponse assay in ovarian cancer. *Clin Transl Oncol* **16**, 761-9 (2014).
89. Schrag, D. et al. American Society of Clinical Oncology Technology Assessment: chemotherapy sensitivity and resistance assays. *J Clin Oncol* **22**, 3631-8 (2004).
90. Aparicio, S., Hidalgo, M. & Kung, A.L. Examining the utility of patient-derived xenograft mouse models. *Nat Rev Cancer* **15**, 311-6 (2015).
91. Byrne, A.T. et al. Interrogating open issues in cancer precision medicine with patient-derived xenografts. *Nat Rev Cancer* **17**, 254-268 (2017).
92. Boj, S.F. et al. Organoid models of human and mouse ductal pancreatic cancer. *Cell* **160**, 324-38 (2015).
93. Eirew, P. et al. Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. *Nature* **518**, 422-6 (2015).
94. Montero, J. et al. Drug-induced death signaling strategy rapidly predicts cancer response to chemotherapy. *Cell* **160**, 977-89 (2015).
95. Montero, J. & Letai, A. Dynamic BH3 profiling-poking cancer cells with a stick. *Mol Cell Oncol* **3**, e1040144 (2016).

96. Montero, J. et al. Blastic Plasmacytoid Dendritic Cell Neoplasm Is Dependent on BCL2 and Sensitive to Venetoclax. *Cancer Discov* **7**, 156-164 (2017).
97. Townsend, E.C. et al. The Public Repository of Xenografts Enables Discovery and Randomized Phase II-like Trials in Mice. *Cancer Cell* **30**, 183 (2016).
98. Ryan, J., Montero, J., Rocco, J. & Letai, A. iBH3: simple, fixable BH3 profiling to determine apoptotic priming in primary tissue by flow cytometry. *Biol Chem* **397**, 671-8 (2016).
99. Frismantas, V. et al. Ex vivo drug response profiling detects recurrent sensitivity patterns in drug-resistant acute lymphoblastic leukemia. *Blood* **129**, e26-e37 (2017).
100. Cermak, N. et al. High-throughput measurement of single-cell growth rates using serial microfluidic mass sensor arrays. *Nat Biotechnol* **34**, 1052-1059 (2016).
101. Godin, M. et al. Using buoyant mass to measure the growth of single cells. *Nat Methods* **7**, 387-90 (2010).
102. Stevens, M.M. et al. Drug sensitivity of single cancer cells is predicted by changes in mass accumulation rate. *Nat Biotechnol* (2016).
103. Cetin, A.E. et al. Determining therapeutic susceptibility in multiple myeloma by single-cell mass accumulation. *Nat Commun* (2017 [*in press*]).
104. Zangle, T.A. & Teitell, M.A. Live-cell mass profiling: an emerging approach in quantitative biophysics. *Nat Methods* **11**, 1221-8 (2014).
105. Byun, S. et al. Characterizing deformability and surface friction of cancer cells. *Proc Natl Acad Sci U S A* **110**, 7580-5 (2013).
106. Gossett, D.R. et al. Hydrodynamic stretching of single cells for large population mechanical phenotyping. *Proc Natl Acad Sci U S A* **109**, 7630-5 (2012).
107. Otto, O. et al. Real-time deformability cytometry: on-the-fly cell mechanical phenotyping. *Nat Methods* **12**, 199-202, 4 p following 202 (2015).
108. Rosenbluth, M.J., Lam, W.A. & Fletcher, D.A. Analyzing cell mechanics in hematologic diseases with microfluidic biophysical flow cytometry. *Lab Chip* **8**, 1062-70 (2008).
109. Wei, W. et al. Single-Cell Phosphoproteomics Resolves Adaptive Signaling Dynamics and Informs Targeted Combination Therapy in Glioblastoma. *Cancer Cell* **29**, 563-573 (2016).
110. Jain, P. et al. Bioluminescence Microscopy as a Method to Measure Single Cell Androgen Receptor Activity Heterogeneous Responses to Antiandrogens. *Sci Rep* **6**, 33968 (2016).
111. Patel, A.P. et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science* **344**, 1396-401 (2014).
112. Dalerba, P. et al. Single-cell dissection of transcriptional heterogeneity in human colon tumors. *Nat Biotechnol* **29**, 1120-7 (2011).
113. Flores-Montero, J. et al. Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. *Leukemia* **31**, 2094-2103 (2017).
114. Lohr, J.G. et al. Genetic interrogation of circulating multiple myeloma cells at single-cell resolution. *Sci Transl Med* **8**, 363ra147 (2016).
115. Kim, S.H. et al. Location of residual viable tumor cells after neoadjuvant chemotherapy: A new concept with high prognostic performance in osteosarcoma. *J Surg Oncol* **115**, 752-759 (2017).

116. Chao, Y.K. et al. Characterization of residual tumours at the primary site in patients with a near pathological complete response after neoadjuvant chemoradiotherapy for oesophageal cancer. *Br J Surg* **103**, 1874-1879 (2016).
117. Chedgy, E.C. et al. Using the neoadjuvant chemotherapy paradigm to develop precision therapy for muscle-invasive bladder cancer. *Urol Oncol* **34**, 469-76 (2016).
118. Tomblyn, M. et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant* **15**, 1143-238 (2009).
119. in New York Times (Syracuse, NY, 1903).
120. Koeffler, H.P. & Leong, G. Preleukemia: one name, many meanings. *Leukemia* **31**, 534-542 (2017).
121. Castven, D. et al. Adverse genomic alterations and stemness features are induced by field cancerization in the microenvironment of hepatocellular carcinomas. *Oncotarget* **8**, 48688-48700 (2017).
122. Hawthorn, L., Lan, L. & Mojica, W. Evidence for field effect cancerization in colorectal cancer. *Genomics* **103**, 211-21 (2014).
123. Joseph, A.W. et al. Molecular etiology of second primary tumors in contralateral tonsils of human papillomavirus-associated index tonsillar carcinomas. *Oral Oncol* **49**, 244-8 (2013).
124. Nonn, L., Ananthanarayanan, V. & Gann, P.H. Evidence for field cancerization of the prostate. *Prostate* **69**, 1470-9 (2009).
125. Izawa, T. et al. Clonality and field cancerization in intraductal papillary-mucinous tumors of the pancreas. *Cancer* **92**, 1807-17 (2001).
126. Acar, O. et al. Determining the origin of synchronous multifocal bladder cancer by exome sequencing. *BMC Cancer* **15**, 871 (2015).
127. Cheng, L. et al. Molecular determinants of tumor recurrence in the urinary bladder. *Future Oncol* **5**, 843-57 (2009).
128. Jones, T.D. et al. Molecular evidence supporting field effect in urothelial carcinogenesis. *Clin Cancer Res* **11**, 6512-9 (2005).
129. Pollyea, D.A. et al. 2-Hydroxyglutarate in IDH mutant acute myeloid leukemia: predicting patient responses, minimal residual disease and correlations with methylcytosine and hydroxymethylcytosine levels. *Leuk Lymphoma* **54**, 408-10 (2013).
130. Adalsteinsson, V.A. et al. Single cells from human primary colorectal tumors exhibit polyfunctional heterogeneity in secretions of ELR+ CXC chemokines. *Integr Biol (Camb)* **5**, 1272-81 (2013).

Box 1. Nomenclature relevant to minimal residual disease. In many contexts where the term "minimal residual disease" (MRD) has been applied, each of the three words is contestable. To begin clarifying, we have generated several acronyms that more precisely define cells and other matter that are currently classified as MRD or used as *de facto* evidence of MRD.

Minimal relapsable cancer (M-REC): Cancer cells that are fully transformed and capable of proliferating into a diagnosable relapse. Thus, these cells have both genetic and functional properties consistent with malignancy.

Minimal non-relapsable cancer (MN-REC): Cancer cells that are incapable of proliferating into a diagnosable relapse. These cells may either have irreversibly differentiated beyond the ability to act as "cancer stem cells," have been damaged by chemotherapy, radiation or other treatment, or otherwise lack the capacity to drive relapse (e.g. due to an inhospitable microenvironment).

Minimal residual precursors (MRPs): In contrast to M-REC, these are non-fully transformed cells that harbour genetic or other alterations that are also present within the tumour cells. Examples include clonal hematopoiesis in patients who achieve CR after leukaemia treatment[120] and dysplastic cells that persist after treatment for many types of localized carcinoma.[121-128]

Minimal residual nucleic acids (MRNAs): Detectable nucleic acids (e.g. cell free DNA) that suggest but do not prove the existence of M-REC, MN-REC and/or MRPs. These may be detected within biopsies or from compartments like the bloodstream, bone marrow, stool or cerebrospinal fluid. They may indicate the persistence of MRPs or could represent nucleic acids that arose from dead or excised tumour cells that have not been fully degraded.

Minimal residual metabolites (MRMs): Similar to MRNAs but would include oncometabolites like 2-hydroxyglutarate[129] as well as metabolites that are generated by malignant or pre-malignant cells and are present either at inappropriate concentrations or within inappropriate compartments.

Table 1. Examples of assay formats that could be applied to measure single-cell response within MRD.
*Some assays could be utilized across the different categories (e.g. aptamers could be utilized in microwell plates to detect RNA transcript abundance rather than proteins). Additional biophysical assays (e.g. measuring deformability) have not been explored in MRD but may be feasible. ‡Some assays like microscopy and SMR are likely to require pre-enrichment of tumour cells from MRD prior to employing the assay. §Some assays like flow cytometry and mass cytometry will vary in the need for pre-enrichment based on the frequency of MRD in the starting population. While these assays could likely characterize MRD at frequencies of 1-in-1,000 to 1-in-10,000 cells, further technical innovation will be necessary for even lower frequencies.

Assay format*	Approach	Pre-enrichmen required
Functional		
Dynamic BH3 Profiling (DBP)	Measure mitochondrial cytochrome C release after exposure to a drug and pro-apoptotic BH3 peptides	Dependent on MRD frequency
Suspended Microchannel Resonator (SMR)‡	Define mass accumulation rate of individual cells after exposure to drug	Yes
Proteomics		
Mass cytometry§	Measure markers of drug response, either specific to target or non-specific markers (e.g. markers of apoptosis)	Dependent on MRD frequency
Flow cytometry§	Same as mass cytometry	Dependent on MRD frequency
Microwell plates	Single cells are seeded into individual wells and secretory, phenotypic or other markers of drug response are quantified, e.g. by antibody detection	Yes
Transcriptomics		
Single-cell RNA-Seq	Transcriptome sequencing of drug-exposed cells to define cell state or programs associated with drug response	Dependent on MRD frequency
Imaging		
Optical microscopy‡	Cells are observed for morphologic features or antibody-based markers of drug response	Yes

Legends

Figure 1. Paradigms for management of MRD. The current approach is to treat patients until they achieve a complete remission (CR). This could be through surgical resection, radiation, chemotherapy or combinations. Patients are then typically observed until they relapse. Instead, minimal residual disease (MRD) could be sampled iteratively, tested for therapeutic susceptibility and treated with the agent(s) identified as most effective by that testing. Once a patient's MRD falls below the minimum detectable threshold (dashed line), treatment could either be stopped, continued indefinitely, or continued for a defined period with curative intent. Potential advantages and disadvantages of targeting MRD are listed and discussed further in the text.

Figure 2. Current paradigm for management of MRD in patients with acute lymphoblastic leukaemia. Induction therapy for acute lymphoblastic leukaemia (ALL) induces morphologic remission (<5% blasts, "complete remission") in most patients. Minimal residual disease (MRD), or small amounts of disease undetectable by standard morphologic review, is often detectable using sensitive MRD-assessment techniques such as flow cytometry and sequencing approaches. The current application of MRD testing is to guide the intensity of consolidation therapy. Patients who achieve undetectable or "low" MRD are assigned to lower intensity therapy. Patients who have "high" levels of MRD are assigned to higher intensity therapy. In each case, the intensity is based on predictions from prior studies of the lowest amount of therapy necessary to eradicate MRD. This probabilistic approach results in undertreatment and overtreatment of some patients. Each panel in the figure illustrates a hypothetical level of MRD in the absence of consolidation therapy (top line) and the same MRD optimally treated with consolidation (bottom line).

Figure 3. Suspended microchannel resonator (SMR) and workflow for the mass accumulation rate (MAR) assay. (**A**). An example of the data collection using the SMR is shown as in Stevens et al.[102] Imatinib was added to cultured BCR-ABL-expressing BaF3 cells at

T=0 and the culture was continuously sampled using a serial SMR [100] for 8 hours. Each cell takes ~20 min to pass through the 12 serial SMRs (sSMRs) = 1st SMR, red = last SMR) and the slope of the resulting growth trajectory is the MAR. Initially, the trajectories have a positive slope, but after 6 hours, the slope approaches zero. Although the imatinib treated cells remained viable for >36 hours before significant induction of apoptosis, the MAR decreases in just a few hours. (**B**) Functional properties such as MAR that are rapidly affected by effective therapeutics and precede longer-term phenotypes (e.g. loss of viability) can be linked to molecular properties by isolating individual cells in wells and performing downstream assays. In this example, MAR is used to identify responding (sensitive) and nonresponding (resistant) cells prior to sc-RNA-seq in order to search for programs and cell states associated with resistance.

Online only information

Competing interests statement

D. M. W. declares that he is a consultant and receives research funding from Novartis, and is a founder of Travera. S. R. M. declares that he is a founder of Affinity Biosensors, and a founder and scientific advisor of Travera. The other authors declare no competing interests.

Table of contents summary

Improved therapies have allowed many patients with cancer to achieve complete remission, but they retain minimal residual disease (MRD) that causes relapse. This Opinion article argues that iterative detection, profiling and targeting of MRD could improve outcomes, including cure rates.

Subject categories

Biological sciences / Cancer / Haematological cancer / Leukaemia
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