OPINION

Targeting minimal residual disease: a path to cure?

Marlise R. Luskin, Mark A. Murakami, Scott R. Manalis and David M. Weinstock

Abstract | 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 radiographical or pathological remission, yet retain minimal residual disease (MRD), which 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 would meaningfully improve outcomes and may even lead to cure.

Complete remission (CR) in patients with cancer is traditionally defined as the absence of a visible tumour by use of sensitive radiological imaging (for example, positron emission tomography (PET) and/or computer tomography (CT) or magnetic resonance imaging (MRI) scan) and, in some cases, histological examination of tissue. Conventional chemotherapy regimens induce CR in the majority of patients with acute leukaemia or aggressive lymphoma[1–4]. By 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), epidermal growth factor receptor (*EGFR*)-mutated or ALK receptor tyrosine kinase (*ALK*)-rearranged lung adenocarcinoma, KIT proto-oncogene receptor tyrosine kinase (*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 is currently under preclinical and clinical investigation. Used as monotherapies and in combination, these agents are likely to usher in a new era in which patients with advanced haematological and solid cancers achieve CR in both front-line and salvage settings.

CR, whether achieved by chemotherapy, targeted therapy, radiation, surgery or a combination, typically requires >99% (that is, >2–3 $\log_{10}$) reduction in tumour burden[17,18]. In a hypothetical patient with five metastatic lesions averaging 2 cm^3 each, this would equate to a reduction from approximately 10^{10} tumour cells to $<10^8$ tumour cells (FIG. 1). These remaining tumour cells have traditionally been called minimal residual disease (MRD). Not all MRD cells are likely to contribute to a clinical relapse, so the term MRD is somewhat nonspecific. Although a full discussion is beyond the scope of this Opinion, we introduce a potentially useful nomenclature to distinguish different types of residual matter in BOX 1.

For the sake of simplicity, we define MRD as follows: malignant cells that remain in a patient who achieves CR and that share phenotypic similarity (for example, histologic appearance and lineage markers) and genetic heritage (for example, 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.

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 with the likelihood of subclonal resistance to one or more therapeutics[19] (FIG. 1). Second, minimal numbers of malignant cells may be less effective at remodelling microenvironments, reprogramming infiltrating haematopoietic 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 substantial side effects may be better when only MRD is present compared with the time of fulminant relapse. Fourth, if cure requires the eradication of all tumour cells capable of driving relapse, there are likely fewer of these cells at the time of MRD. Cancer stem cells are less sensitive to many drugs than more differentiated tumour cells, 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 with the time of frank relapse[21].

Evidence that treating MRD can increase the rates of cure. The strongest evidence that treatment of MRD can prevent relapse, which is a measurable surrogate for cure, comes from the experience of 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 haematological 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].

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 (that is, treatment after achieving CR) on the basis of MRD load is standard practice[47]. The presence or absence of MRD above defined thresholds directs relatively crude de-intensification (that is, lower doses) or intensification (that is, higher doses with or without allogeneic stem cell transplantation) of therapy; it does not guide the selection of targeted therapeutics to exploit the biological vulnerabilities of MRD (FIG. 2). Increasing levels of MRD after completion of therapy can inform clinicians about impending relapse. This may provide more time to plan salvage therapy but, again, does not guide the selection of targeted approaches to eradicate resistant clones.

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 incorporates 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-line or third-line therapy. Patients with CML who achieve optimal responses to first-line or second-line TKI therapy (defined as sustained, deep remissions of greater than 4–5 $\log_{10}$ reductions in the *BCR–ABL* transcript according to 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 discontinued therapy under those circumstances remained in prolonged molecular remission[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 had 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

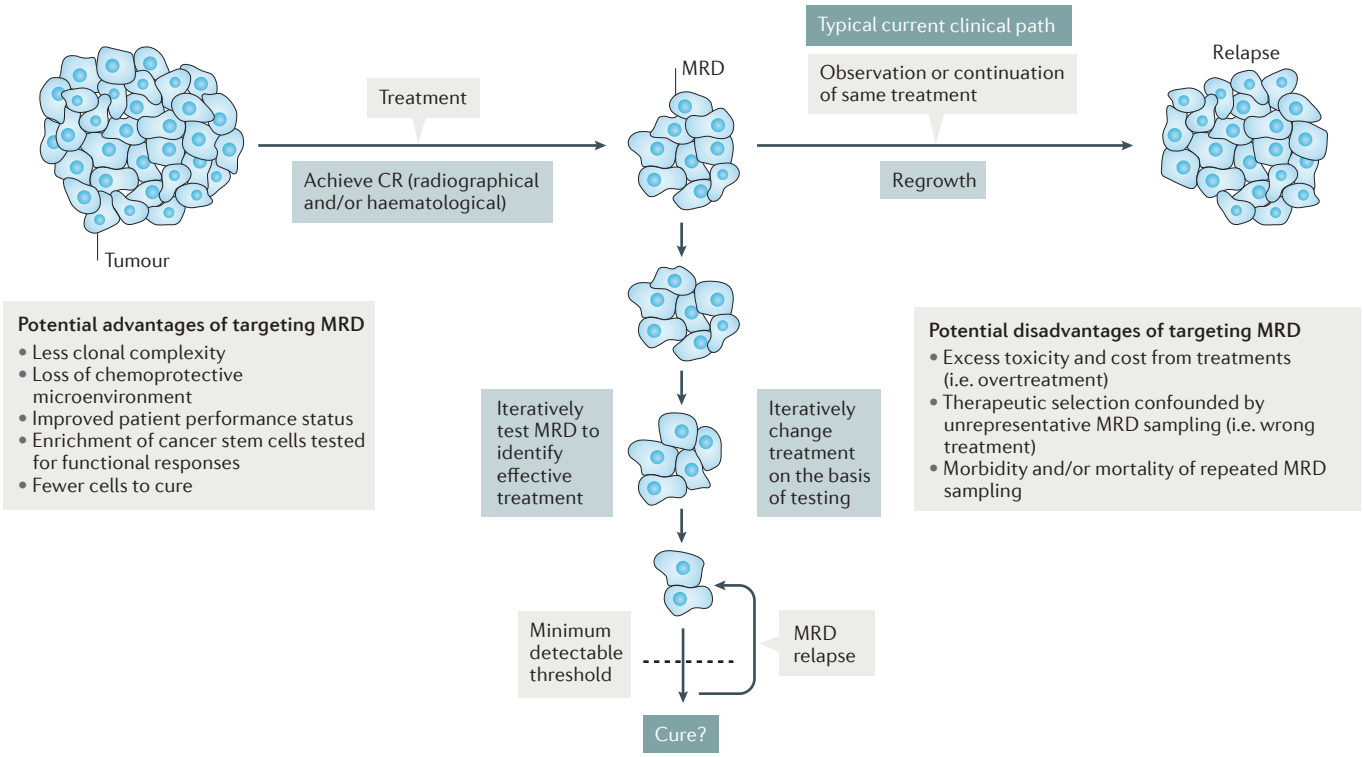

Figure 1 | **Paradigms for management of MRD.** The current approach is to treat patients until they achieve complete remission (CR). This could be through surgical resection, radiation, chemotherapy or a combination of these treatments. Patients are then typically observed until they relapse. Instead, minimal residual disease (MRD) could be sampled iteratively, tested for therapeutic susceptibility and treated with the agents identified as most effective by that test. Once a patient's MRD falls below the minimum detectable threshold (dashed line), treatment could 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.

malignant cells. The reasons for this remain poorly understood, but one possibility is that CML stem cells are primarily quiescent and only intermittently enter the 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 the 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 might 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 2–3 years[61,62]. Resistant clones that emerge late in this maintenance phase frequently harbour mutations in 5′-nucleotidase, cytosolic II (*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 (that is, 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-line 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 (for example, by genetic loss of genes encoding apoptosis effectors[65]).

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. 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 have irreversibly differentiated beyond the ability to act as cancer stem cells, may have been damaged by chemotherapy, radiation or other treatment, or may otherwise lack the capacity to drive relapse (for example, owing 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 haematopoiesis in patients who achieve complete remission after leukaemia treatment[121] and dysplastic cells that persist after treatment for many types of localized carcinomas[122–129].

Minimal residual nucleic acids (MRNAs). Detectable nucleic acids that suggest, but do not prove, the existence of M-REC, MN-REC and/or MRPs. These may be detected within biopsy samples or from compartments such as 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). Metabolites that are similar to MRNAs but would include oncometabolites such as 2-hydroxyglutarate[130] as well as metabolites that are generated by malignant or pre-malignant cells and are present either at inappropriate concentrations or within inappropriate compartments.

Approaches to characterize MRD

Biological and functional challenges. An optimal assay performed at the time of CR would not only predict therapeutic response but 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 to guide therapeutic selection (as in FIG. 1). This would be a transformative clinical advance for patients with persistent MRD if selective targeting of 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 derived 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 haematological cancers. For example, independent metastases from the same carcinoma can undergo divergent evolution that leads to differential therapeutic responses, and even primary solid tumours can have extensive geographic heterogeneity[66–68]. There is a widely held belief that haematological cancers may be less geographically heterogeneous than carcinomas, but this has not been systematically proved. The larger advantage for sampling MRD in patients with leukaemias and some lymphomas is that MRD cells can be isolated from the bone marrow. By 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: there is an inadequate signal:noise ratio for the detection of MRD by use of currently available imaging approaches, and biopsy of sites such as 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 use 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, such as apheresis, 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 such as apheresis may be merited if therapeutic selection based on a predictive assay performed using that material considerably improved patient outcome. Alternatively, agents that interrupt tumour cell interactions with the microenvironment, such as those targeting CXC-chemokine receptor 4 (CXCR4) or E-selectin, could be used to transiently increase the number of CTCs in a patient with MRD[73]; importantly, concerns have been raised that this type of approach may increase metastasis[74]. Finally, one could imagine testing multiple different sites of MRD from a patient with micrometastases if technological advances allowed for identification of those sites (for example, with new imaging strategies) and minimally invasive sampling (for example, with microneedles).

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[75–79]. In tumours where a targeted agent is available and associated with genotype-specific activity (that is, 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[80].

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 focus primarily on phenotypic assays, as they capture complex and multiparametric interactions to predict *in vivo* therapeutic efficacy. However, there are several transcriptional and proteomic 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, post-translational modifications and proteolysis products, from millions of single cells within an individual sample[81]. Mass cytometry can reveal cell signalling programmes as well as markers of intercellular communication (for example, cytokines or growth factors) that reflect the surrounding environment. Single-cell RNA sequencing (scRNA-seq) is similarly capable of defining multiple aspects of the cell state as well as genotype[82]. Microfluidic approaches have now made it possible to conduct scRNA-seq or other sequencing platforms on thousands of cells at low cost[83–85], revealing intratumoural heterogeneity in both malignant and non-malignant populations[83,86,87]. However, these vast insights into tumour biology have not thus far been translated into clinically relevant approaches for selecting therapeutics for an individual patient[80].

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[88–90]. Tumour cells were plated in 2D culture *ex vivo* in the presence

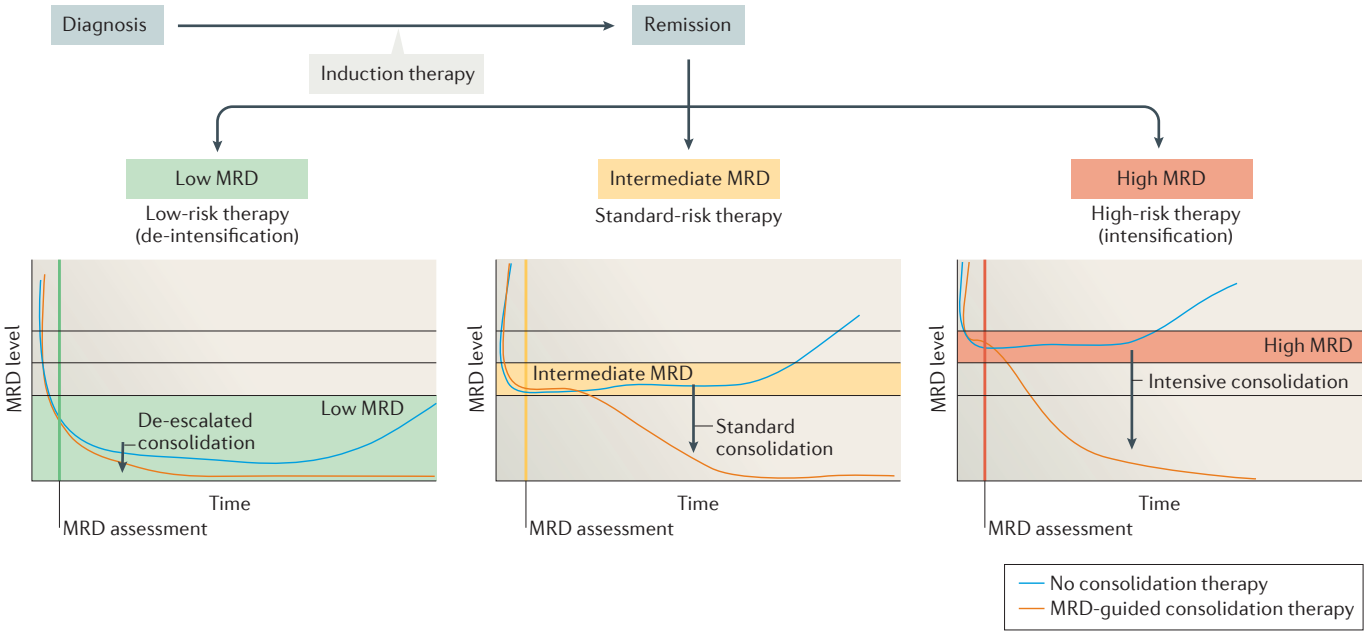

Figure 2 | **Current paradigm for management of MRD in patients with acute lymphoblastic leukaemia.** Induction therapy for acute lymphoblastic leukaemia induces morphologic remission (<5% blasts, complete remission) in most patients. Minimal residual disease (MRD), or small amounts of disease undetectable by standard morphological review, is often detectable by use of 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 (blue line) and the same MRD optimally treated with consolidation (orange line).

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 notable 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 2D 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[91–94].

An alternative approach is to either eliminate or reduce the need for *ex vivo* propagation through the use of 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. A non-exhaustive 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[95]. In DBP, patient tumour cells are exposed to drugs for 16–24 hours *ex vivo*, permeabilized and exposed to pro-apoptotic, 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[96]. DBP has been used to accurately predict the response of CML to imatinib therapy by use of bone marrow samples, the response of single-cell suspensions from ovarian cancer biopsies to carboplatin therapy, the response of ALL PDXs to E3 ubiquitin-protein ligase MDM2 inhibition and in other settings[95,97,98].

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 2D culture, organoids and PDXs, DBP queries the treatment response of tumour cells in bulk rather than in individual cells, although advances in flow cytometry-based DBP may allow for its application to single cells[99]. Other approaches that utilize flow cytometry could also be applied to assay the effects of therapeutics when exposed to samples *ex vivo*[100]. 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 and the Dana-Farber Cancer Institute, 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 (FIG. 3). When an object denser than media passes through the SMR, the net increase in mass (that is, the buoyant mass of the object) lowers the resonant frequency. To measure the mass accumulation rate (MAR) of a cell, an array of SMRs are microfluidically connected

Table 1 | **Formats for measuring single-cell response in MRD**

Assay format*	Approach	Pre-enrichment required	Destructive	Refs
Functional				
DBP	Measures mitochondrial cytochrome C release after exposure to a drug and pro-apoptotic BH3 peptides	Dependent on MRD frequency	Yes	95–99
SMR‡	Defines mass accumulation rate of individual cells after exposure to drug	Yes	No	101–103
Proteomics				
Mass cytometry§	Measures markers of drug response, either specific to target or nonspecific markers (for example, markers of apoptosis)	Dependent on MRD frequency	Yes	81
Flow cytometry§	Same as mass cytometry	Dependent on MRD frequency	Yes, if using intracellular markers	100
Microwell plates	Single cells are seeded into individual wells, and secretory, phenotypic or other markers of drug response are quantified, for example, by antibody detection	Yes	Yes, if using intracellular markers	110,131
Transcriptomics				
Single-cell RNA-seq	Transcriptome sequencing of drug-exposed cells to define cell state or programmes associated with drug response	Dependent on MRD frequency	Yes	82–87
Imaging				
Optical microscopy‡	Cells are observed for morphological features or antibody-based markers of drug response	Yes	No	106

The formats listed in the table are an incomplete list of example technologies. DBP, dynamic BH3 profiling; MRD, minimal residue disease; RNA-seq, RNA sequencing; SMR, suspended microchannel resonator. *Some assays could be used across the different categories (for example, aptamers could be used in microwell plates to detect RNA transcript abundance rather than proteins). Additional biophysical assays (for example, measuring deformability) have not been explored in MRD but may be feasible. ‡Some assays, such as microscopy and SMR, are likely to require pre-enrichment of tumour cells from MRD before employing the assay. §Some assays, such as 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.

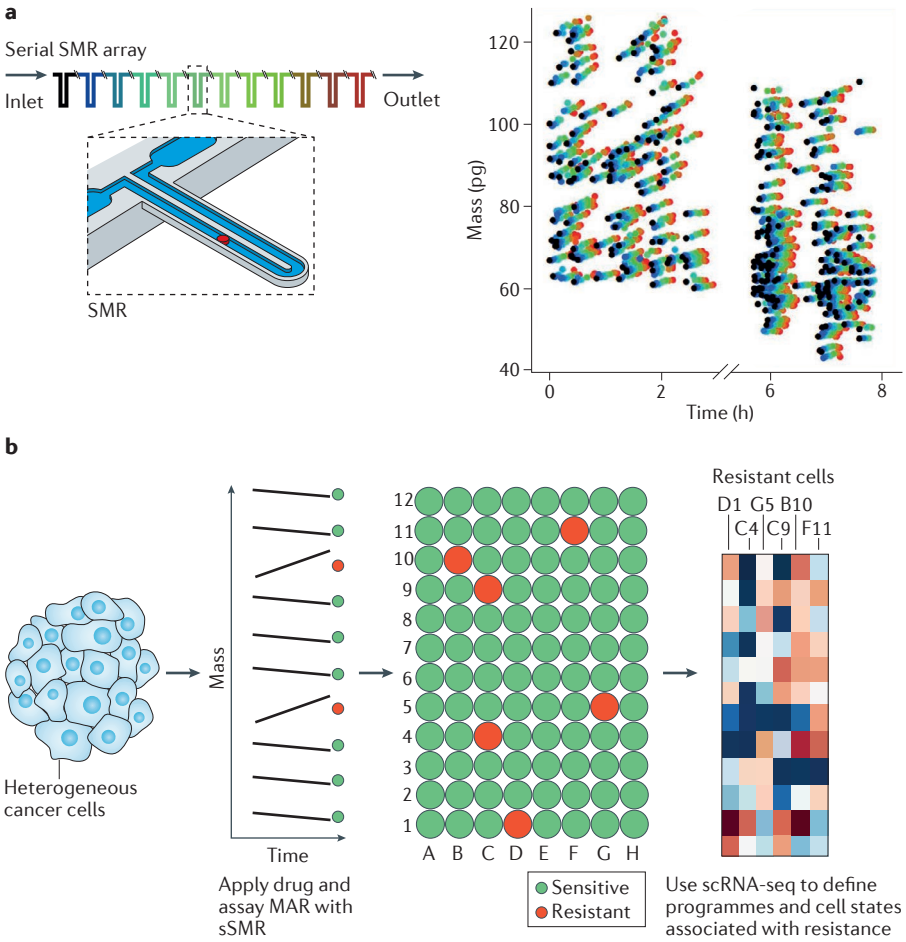

Figure 3 | **Suspended microchannel resonator and workflow for the mass accumulation rate assay. a** | An example of data collection by use of the suspended microchannel resonator (SMR) is shown, which is analogous to data published previously in Stevens *et al.*[103]. Imatinib was added to cultured BCR–ABL-expressing BaF3 cells at $T = 0$, and the culture was continuously sampled using a serial SMR[101] for 8 hours. Each cell takes ~20 minutes to pass through the 12 serial SMRs (sSMRs) (black = first SMR, red = last SMR), and the slope of the resulting growth trajectory is the mass accumulation rate (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 measurable 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 (for example, 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 before single-cell RNA sequencing (scRNA-seq) in order to search for programmes and cell states associated with resistance. Part **a** courtesy of N. Cermak.

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[101,102]. 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*[103].

We have demonstrated that the MAR assay can define the drug sensitivity or resistance of glioblastoma and B cell ALL cells to targeted agents[103]. 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 six patients who responded to therapy that included the proteasome inhibitor bortezomib and three who did not, MAR correctly predicted response with 100% accuracy[104]. 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 (FIG. 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, pretreatment and MRD samples can be compared to define the effects of therapy on clonal architecture. We envision that 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[105], and several different microfluidic approaches have been used to interrogate mechanical properties of single cells[106–109]. Label-independent techniques that exploit differences in the size, shape or rigidity of malignant versus non-malignant cells also exist but have not yet been fully exploited for identifying MRD cells.

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 by use of antibodies conjugated to magnetic beads or fluorescent molecules followed by either flow-associated cell sorting or positive selection with magnetic beads[110–113].

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 approximately two MRD cells per million[114]. Although identification of MRD at this frequency is now possible, sorting and then drug testing these cells are unlikely to be possible. However, testing frequencies of MRD between 1 in 10,000 and 1 in 1,000 cells should be feasible based on

the ability to sequence individual cells present at even lower frequencies[115]. 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 by using carcinoma and sarcoma resections after neoadjuvant therapy[116–118].

The future of MRD-guided therapy

It remains to be determined whether applying any approach to MRD samples can lead to the selection of treatments that extend the duration of CR over current empirical 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 proved 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. The achievement of 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 FIG. 1). At that time, MRD would be assessed for therapeutic sensitivity and the appropriate treatment initiated (or re-initiated). This is a form of preemptive therapy similar to the suppression of viral reactivation among immunocompromised patients[119]. 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 haematological 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 receive either the treatment predicted to be optimal based on MRD testing or the treating physician's choice of therapy. Upon treatment failure (either upfront failure or failure 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 (that is, 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) end points 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[120], "Far and away the best prize that life offers is the chance to work hard at work worth doing."

Marlise R. Luskin and Mark A. Murakami are at the Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts 02215, USA.

Scott R. Manalis is at the Koch Institute for Integrative Cancer Research and the Departments of Biological Engineering and Mechanical Engineering at the Massachusetts Institute of Technology, Cambridge, Massachusetts 02139,USA.

David M. Weinstock is at the Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts 02215, USA, and at the Broad Institute of the Massachusetts Institute of Technology and Harvard University, Cambridge, Massachusetts 02142, USA.

Correspondence to S.R.M. and D.M.W.
srm@mit.edu; davidm_weinstock@dfci.harvard.edu

doi:10.1038/nrc.2017.125
Published online 29 Jan 2018

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**, 3604–3610 (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**, 90–94 (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**, 3543–3551 (2008).
6. Souglakos, J. *et al.* FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) versus 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**, 314–321 (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**, 2745–2751 (1999).
9. Casper, E. S. Gastrointestinal stromal tumors. *Curr. Treat. Opt. Oncol.* **1**, 267–273 (2000).
10. Tannock, I. F. *et al.* Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N. Engl. J. Med.* **351**, 1502–1512 (2004).
11. Maemondo, M. *et al.* Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N. Engl. J. Med.* **362**, 2380–2388 (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**, 992–999 (2015).
14. Long, G. V. *et al.* Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N. Engl. J. Med.* **371**, 1877–1888 (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**, 1248–1260 (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**, 626–632 (2008).
17. Hourigan, C. S. & Karp, J. E. Minimal residual disease in acute myeloid leukaemia. *Nat. Rev. Clin. Oncol.* **10**, 460–471 (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. USA* **112**, 8843–8850 (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**, 30–36 (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**, 1879–1887 (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**, 3109–3116 (2009).
25. Early Breast Cancer Trialists' Collaborative Group. 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**, 432–444 (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**, 573–581 (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* http://dx.doi.org/10.1007/s12282-017-0794-8 (2017).
28. Winton, T. *et al.* Vinorelbine plus cisplatin versus observation in resected non-small-cell lung cancer. *N. Engl. J. Med.* **352**, 2589–2597 (2005).
29. Frei, E. *et al.* The effectiveness of combinations of antileukemic agents in inducing and maintaining remission in children with acute leukemia. *Blood* **26**, 642–656 (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**, 123–136 (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? *Hematol. Am. Soc. Hematol. Educ. Program* **2016**, 356–365 (2016).
34. Campana, D. Minimal residual disease in acute lymphoblastic leukemia. *Hematol. 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**, 1116–1123 (2006).
36. Bruggemann, M., Raff, T. & Kneba, M. Has MRD monitoring superseded other prognostic factors in adult ALL? *Blood* **120**, 4470–4481 (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**, 3650–3658 (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**, 2826–2835 (2012).
39. Beldjord, K. *et al.* Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. *Blood* **123**, 3739–3749 (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**, 5477–5485 (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.* **33**, 1258–1264 (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**, 4123–4131 (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**, 1868–1876 (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**, 3889–3897 (2013).
45. Cave, H. *et al.* Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. *N. Engl. J. Med.* **339**, 591–598 (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**, 4695–4700 (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**, 2591–2601 (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**, 1214–1221 (2013).
49. Cortes, J. & Kantarjian, H. How I treat newly diagnosed chronic phase CML. *Blood* **120**, 1390–1397 (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**, 515–522 (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**, 424–430 (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**, e528–535 (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**, 197–199 (2007).
57. Michor, F. *et al.* Dynamics of chronic myeloid leukaemia. *Nature* **435**, 1267–1270 (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**, 4016–4019 (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**, 767–775 (2012).
61. Lonsdale, D. *et al.* Interrupted versus continued maintenance therapy in childhood acute leukemia. *Cancer* **36**, 341–352 (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**, 1289–1295 (1993).
63. Tzoneva, G. *et al.* Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL. *Nat. Med.* **19**, 368–371 (2013).
64. Meyer, J. A. *et al.* Relapse-specific mutations in NT5C2 in childhood acute lymphoblastic leukemia. *Nat. Genet.* **45**, 290–294 (2013).
65. Meijerink, J. P. *et al.* Hematopoietic malignancies demonstrate loss-of-function mutations of BAX. *Blood* **91**, 2991–2997 (1998).
66. Juric, D. *et al.* Convergent loss of PTEN leads to clinical resistance to a PI(3)Kalpha inhibitor. *Nature* **518**, 240–244 (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**, 6302–6309 (2008).
72. Fischer, J. C. *et al.* Diagnostic leukapheresis enables reliable detection of circulating tumor cells of nonmetastatic cancer patients. *Proc. Natl Acad. Sci. USA* **110**, 16580–16585 (2013).
73. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02478125 (2017)
74. 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).
75. Wan, J. C. *et al.* Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat. Rev. Cancer* **17**, 223–238 (2017).
76. 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**, 1345–1353 (2014).
77. 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**, 1682–1689 (2016).
78. 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**, 1014–1022 (2016).
79. 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).
80. Letai, A. Functional precision cancer medicine-moving beyond pure genomics. *Nat. Med.* **23**, 1028–1035 (2017).
81. Spitzer, M. H. & Nolan, G. P. Mass cytometry: single cells, many features. *Cell* **165**, 780–791 (2016).
82. Shalek, A. K. & Benson, M. Single-cell analyses to tailor treatments. *Sci. Transl Med.* **9**, eaan4730 (2017).
83. Gierahn, T. M. *et al.* Seq-Well: portable, low-cost RNA sequencing of single cells at high throughput. *Nat. Methods* **14**, 395–398 (2017).
84. Klein, A. M. *et al.* Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell* **161**, 1187–1201 (2015).
85. Macosko, E. Z. *et al.* Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell* **161**, 1202–1214 (2015).
86. 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).
87. Tirosh, I. *et al.* Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* **352**, 189–196 (2016).
88. Friedman, A. A., Letai, A., Fisher, D. E. & Flaherty, K. T. Precision medicine for cancer with next-generation functional diagnostics. *Nat. Rev. Cancer* **15**, 747–756 (2015).
89. Grendys, E. C. *et al.* Overview of a chemoresponse assay in ovarian cancer. *Clin. Transl Oncol.* **16**, 761–769 (2014).
90. Schrag, D. *et al.* American Society of Clinical Oncology Technology Assessment: chemotherapy sensitivity and resistance assays. *J. Clin. Oncol.* **22**, 3631–3638 (2004).
91. Aparicio, S., Hidalgo, M. & Kung, A. L. Examining the utility of patient-derived xenograft mouse models. *Nat. Rev. Cancer* **15**, 311–316 (2015).
92. Byrne, A. T. *et al.* Interrogating open issues in cancer precision medicine with patient-derived xenografts. *Nat. Rev. Cancer* **17**, 254–268 (2017).
93. Boj, S. F. *et al.* Organoid models of human and mouse ductal pancreatic cancer. *Cell* **160**, 324–338 (2015).
94. Eirew, P. *et al.* Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. *Nature* **518**, 422–426 (2015).
95. Montero, J. *et al.* Drug-induced death signaling strategy rapidly predicts cancer response to chemotherapy. *Cell* **160**, 977–989 (2015).
96. Montero, J. & Letai, A. Dynamic BH3 profiling-poking cancer cells with a stick. *Mol. Cell Oncol.* **3**, e1040144 (2016).
97. Montero, J. *et al.* Blastic plasmacytoid dendritic cell neoplasm is dependent on BCL2 and sensitive to venetoclax. *Cancer Discov.* **7**, 156–164 (2017).

98. 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).
99. 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**, 671–678 (2016).
100. Frismantas, V. *et al. Ex vivo* drug response profiling detects recurrent sensitivity patterns in drug-resistant acute lymphoblastic leukemia. *Blood* **129**, e26–e37 (2017).
101. Cermak, N. *et al.* High-throughput measurement of single-cell growth rates using serial microfluidic mass sensor arrays. *Nat. Biotechnol.* **34**, 1052–1059 (2016).
102. Godin, M. *et al.* Using buoyant mass to measure the growth of single cells. *Nat. Methods* **7**, 387–390 (2010).
103. Stevens, M. M. *et al.* Drug sensitivity of single cancer cells is predicted by changes in mass accumulation rate. *Nat. Biotechnol.* **34**, 1161–1167 (2016).
104. Cetin, A. E. *et al.* Determining therapeutic susceptibility in multiple myeloma by single-cell mass accumulation. *Nat. Commun.* **8**, 1613 (2017).
105. Zangle, T. A. & Teitell, M. A. Live-cell mass profiling: an emerging approach in quantitative biophysics. *Nat. Methods* **11**, 1221–1228 (2014).
106. Byun, S. *et al.* Characterizing deformability and surface friction of cancer cells. *Proc. Natl Acad. Sci. USA* **110**, 7580–7585 (2013).
107. Gossett, D. R. *et al.* Hydrodynamic stretching of single cells for large population mechanical phenotyping. *Proc. Natl Acad. Sci. USA* **109**, 7630–7635 (2012).
108. Otto, O. *et al.* Real-time deformability cytometry: on-the-fly cell mechanical phenotyping. *Nat. Methods* **12**, 199–202 (2015).
109. Rosenbluth, M. J., Lam, W. A. & Fletcher, D. A. Analyzing cell mechanics in hematologic diseases with microfluidic biophysical flow cytometry. *Lab Chip* **8**, 1062–1070 (2008).
110. Wei, W. *et al.* Single-cell phosphoproteomics resolves adaptive signaling dynamics and informs targeted combination therapy in glioblastoma. *Cancer Cell* **29**, 563–573 (2016).
111. 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).
112. Patel, A. P. *et al.* Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science* **344**, 1396–1401 (2014).
113. Dalerba, P. *et al.* Single-cell dissection of transcriptional heterogeneity in human colon tumors. *Nat. Biotechnol.* **29**, 1120–1127 (2011).
114. 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).
115. Lohr, J. G. *et al.* Genetic interrogation of circulating multiple myeloma cells at single-cell resolution. *Sci. Transl Med.* **8**, 363ra147 (2016).
116. 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).
117. 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).
118. Chedgy, E. C. *et al.* Using the neoadjuvant chemotherapy paradigm to develop precision therapy for muscle-invasive bladder cancer. *Urol. Oncol.* **34**, 469–476 (2016).
119. Tomblyn, M. *et al.* Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol. Blood Marrow Transplant* **15**, 1143–1238 (2009).
120. Roosevelt, T. *Address to the New York State Agricultural Association* (Syracuse, 1903).
121. Koeffler, H. P. & Leong, G. Preleukemia: one name, many meanings. *Leukemia* **31**, 534–542 (2017).
122. 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).
123. Hawthorn, L., Lan, L. & Mojica, W. Evidence for field effect cancerization in colorectal cancer. *Genomics* **103**, 211–221 (2014).
124. Joseph, A. W. *et al.* Molecular etiology of second primary tumors in contralateral tonsils of human papillomavirus-associated index tonsillar carcinomas. *Oral Oncol.* **49**, 244–248 (2013).
125. Nonn, L., Ananthanarayanan, V. & Gann, P. H. Evidence for field cancerization of the prostate. *Prostate* **69**, 1470–1479 (2009).
126. Izawa, T. *et al.* Clonality and field cancerization in intraductal papillary-mucinous tumors of the pancreas. *Cancer* **92**, 1807–1817 (2001).
127. Acar, O. *et al.* Determining the origin of synchronous multifocal bladder cancer by exome sequencing. *BMC Cancer* **15**, 871 (2015).
128. Cheng, L. *et al.* Molecular determinants of tumor recurrence in the urinary bladder. *Future Oncol.* **5**, 843–857 (2009).
129. Jones, T. D. *et al.* Molecular evidence supporting field effect in urothelial carcinogenesis. *Clin. Cancer Res.* **11**, 6512–6519 (2005).
130. 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**, 408–410 (2013).
131. Adalsteinsson, V. A. *et al.* Single cells from human primary colorectal tumors exhibit polyfunctional heterogeneity in secretions of ELR + CXC chemokines. *Integr. Biol.* **5**, 1272–1281 (2013).

Acknowledgements
The authors thank C. Love and M. Stevens for thoughtful review and comments. D.M.W. and S.R.M. are supported by the Koch Institute–Dana-Farber/Harvard Cancer Center Bridge Project, the National Cancer Institute (NCI) R33 CA191143 and the NCI Cancer Systems Biology Consortium U54 CA217377. D.M.W. is a Leukaemia and Lymphoma Society Scholar. M.A.M. is supported by NCI K08 CA212252.

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.