A view on drug resistance in cancer

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The problem of resistance to therapy in cancer is multifaceted. Here we take a reductionist approach to define and separate the key determinants of drug resistance, which include tumour burden and growth kinetics; tumour heterogeneity; physical barriers; the immune system and the microenvironment; undruggable cancer drivers; and the many consequences of applying therapeutic pressures. We propose four general solutions to drug resistance that are based on earlier detection of tumours permitting cancer interception; adaptive monitoring during therapy; the addition of novel drugs and improved pharmacological principles that result in deeper responses; and the identification of cancer cell dependencies by high-throughput synthetic lethality screens, integration of clinico-genomic data and computational modelling. These different approaches could eventually be synthesized for each tumour at any decision point and used to inform the choice of therapy.

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Drug resistance continues to be the principal limiting factor to achieving cures in patients with cancer. The problem of drug resistance in cancer has strong similarities to the field of infectious disease, in that both disciplines are challenged by highly proliferating intrinsic or extrinsic aggressors. As with antimicrobial therapy, the excitement that was brought about by initial successes of early chemotherapeutics (such as nitrogen mustard[1] and aminopterin[2]) was quickly tempered by evidence showing that although tumours went into remission quickly, they developed resistance, resulting in disease relapse.

The initial solution to the problem of resistance to single-agent chemotherapy—the combined administration of agents with non-overlapping mechanisms of action, or polychemotherapy—was taken, unsurprisingly, from the rulebook of antimicrobial therapy[3]. This empirical approach worked remarkably well in some forms of lymphoma, breast cancer and testicular cancer[4–6]. Combination chemotherapy thus became a new paradigm for cancer therapy that led to the development of increasingly complex regimens. In addition, a number of different approaches to dose intensity[7], including shorter-interval administrations of chemotherapy[8,9] or higher doses of chemotherapy[9] with growth factor support to prevent continued bone marrow suppression, resulted in improved success of these therapies by preventing early regrowth of tumours. At the turn of the century, almost 50 years after its introduction, the successes achieved with polychemotherapy had largely plateaued. Surgery, radiotherapy and polychemotherapy were clearly not enough to cure many tumour types.

As a result, new therapeutic strategies directed at targeting the key enabling characteristics and acquired capabilities that transform normal cells and tissues into malignancies began to be developed. The introduction of therapies that disrupted these hallmark features[10,11], including targeted therapies, was an important leap forward. Indeed, the understanding of the biological determinants of cancer has resulted in highly efficacious therapies against tyrosine kinases, nuclear receptors and other molecular targets. The initial successes of oestrogen receptor (ER) and androgen receptor (AR) antagonists, as well as BCR-ABL, HER2 and EGFR inhibitors, led to a massive effort to develop agents that target oncogenes and other key cellular vulnerabilities. More recently, oncological therapy has advanced again by using immunological approaches to recognize and attack cancer. Anti-CTLA4[12] and anti-PD-1/PD-L1[13] monoclonal antibodies that disable negative regulators, or checkpoints, of the adaptive immune system have resulted in remarkable anti-tumour activity—and even cures—in multiple tumour types[14]. And yet, similarly to what was previously observed with conventional chemotherapy, eventual resistance to targeted and immunological therapies remains the norm. This is where the similarities between cancer and infectious diseases may dissociate: combination therapy often leads, for example, to disease becoming undetectable in HIV or cured in tuberculosis, but in metastatic cancers this is the exception rather than the rule[15]. Not unexpectedly, cancer appears to be a more-complex biological problem.

Here we attempt to present a framework for conceptualizing drug resistance in cancer by enumerating the basic determinants of resistance and considering their implications for the development of successful therapeutic strategies. These basic determinants of resistance, which are present in unique iterations during the history of a cancer, result in different clinical states that range from extreme sensitivity to complete resistance to therapy. We describe standard and emerging interventions that target these determinants, and consider how new technological and pharmacological advances can be integrated with these interventions to prevent, delay or revert resistance to therapy.

Biological determinants of resistance

In its simplest definition, cancer therapy operates as a three-component system: (i) a therapy; that targets (ii) a population of cancer cells; within

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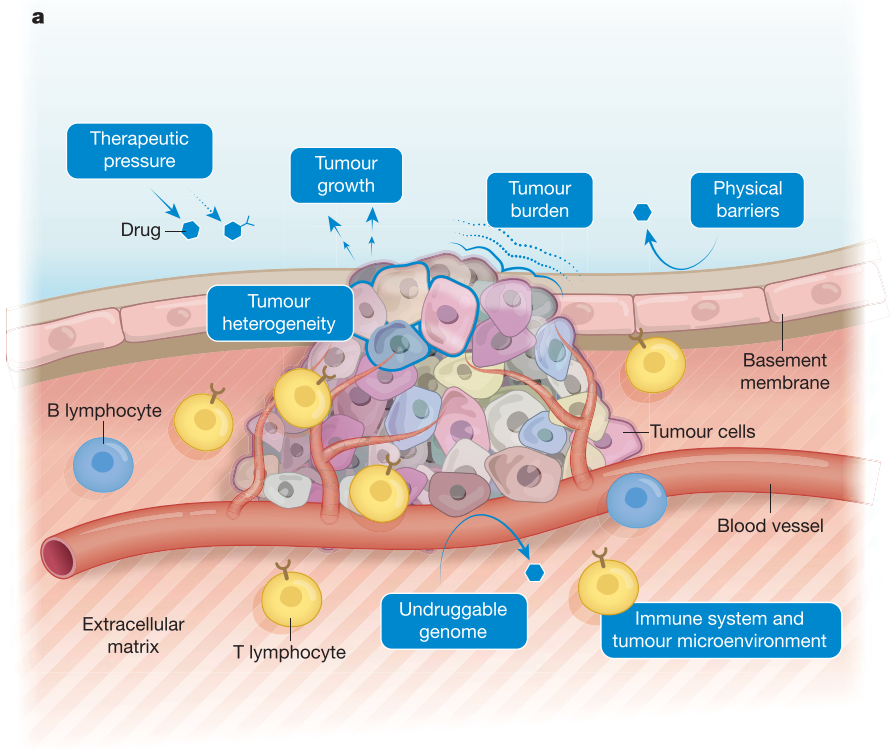

Fig. 1 | A framework for understanding drug resistance. a, Biological determinants of drug resistance. Tumours are heterogeneous and are situated in a milieu that comprises the basement membrane, vasculature, immune cells and tumour microenvironment, among other components. Changes in the physical parameters, genome and surrounding environment of the tumour drive drug resistance. **b**, Standard of care and emerging approaches to managing the biological determinants of resistance. The determinants of resistance can be targeted by a number of clinical diagnostic and therapeutic strategies.

(iii) a particular host environment. The pharmacological properties of the therapy, together with intrinsic and acquired physical and molecular parameters of cancer cells and extrinsic environmental factors, result in the spectrum of clinical responses. Many descriptions of drug resistance in cancer have focused on the binary differences between intrinsic and acquired resistance; however, in practice, many tumours are or become resistant owing to overlapping combinations of these factors. We propose that, in defining the fundamental biological principles of resistance, a framework can be created for understanding resistance both to existing and future therapies (Fig. 1a). We believe that by focusing both cancer and clinical science on addressing each determinant separately, the resistance problem can be managed (Fig. 1b). The biological determinants of resistance are delineated in the next sections.

Tumour burden and growth kinetics

There is an almost universal correlation between tumour burden and curability[16]. In many tumour types, the size of the tumour (or number of cells, in the case of liquid tumours) at diagnosis is perhaps the most frequently used variable to estimate prognosis; larger tumours correlate with increased metastatic risk[17]. This inverse correlation between size and curability was not entirely anticipated in the infancy of chemotherapy. Early mathematical models, such as the 'log kill' hypothesis, proposed that combining multiple drugs that individually kill a logarithmic fraction of cells over multiple cycles would permit sequential decreases in tumour burden until the disease was fully eradicated[18]. This is true in tumours that are highly sensitive to chemotherapy, such as some lymphomas and germ cell tumours, but does not hold across many other cancer types.

To more accurately model cancer growth, the Goldie–Coldman hypothesis was proposed[19]. This model, which was informed by seminal microbiology experiments[20], accounts for tumour size and also incorporates the emergence of resistance. According to this hypothesis, the probability that a cancer contains drug-resistant clones depends on the mutation rate and the size of the tumour[19]. In fact, given a certain mutation rate, size becomes the key determinant in predicting the presence of drug-resistant mutations. Multiple concepts have stemmed from this model, including the notion that alternating non-cross-resistant combinations of chemotherapy, rather than administering all therapies at once (which is often limited by toxicity), is superior in preventing drug resistance as compared to sequential therapies. Alternation of therapy sequences would allow the tumour to be exposed to a greater number of total drugs by an earlier time point. This hypothesis has not been uniformly borne out in clinical practice, suggesting that there are additional complexities that need to be considered[21].

Moreover, although tumour size is a critical determinant of resistance, the rate of tumour growth and the changes in growth kinetics that are induced by therapy also have a critical role in responses to therapy and resistance. Tumour growth kinetics are highly variable, ranging from indolent to aggressive. Although tumours with low rates of growth are often associated with long survival, they are typically incurable with cytotoxic chemotherapy or even with targeted therapies. By contrast, tumours that grow at higher speeds can be exquisitely sensitive to chemotherapy. There is also a direct relationship between growth rate and tumour size, which explains, for example, the frequency of interval cancer cases in screening programs.

The model that perhaps best explains the growth of cancer and its regression after therapy is the Norton–Simon hypothesis[22]. This model, which applies to most solid tumours, is based on Gompertzian growth curves. According to the model, tumours grow in a sigmoidal manner—exponentially faster at low tumour burdens and subsequently

approaching a plateau with slower growth rates as they reach a larger size[23]. Because drugs reduce the size of tumours, they affect growth kinetics. After a single administration of chemotherapy, the remaining tumour fractions may resume their early phase of exponential growth. Following this logic, the probability of eradication is maximized by preventing rapid regrowth of the tumour between treatments[24]. This led to the concept of dose-dense chemotherapy, an approach in which the most effective dose level of a drug is given over as short a time interval as possible. Clinical proof of concept of dose density has been demonstrated in early breast and ovarian cancer, for which chemotherapy that is administered more frequently has in select circumstances improved overall survival[8,25,26]. Dose-dense approaches have not been sufficient to convert ineffective therapies into effective ones, but rather have been used to improve the efficacy of established approaches. Less attention has been paid to the role of dose scheduling in targeted therapies than in chemotherapy, and we propose that this could be a strategy to deepen responses and increase cures.

Tumour heterogeneity

Cancer heterogeneity is perhaps the cause of drug resistance that is easiest to conceptualize[27]. Cancer cells acquire genomic alterations through a variety of mutational processes that generate spatial and temporal genetic diversity[28]. These processes occur at different evolutionary speeds—from the relatively slow rate of age-related mutations, to frequent editing of genes by APOBEC enzymes (a process that increases over the course of tumour evolution), to bursts of dramatic and catastrophic events that are induced by genomic instability[29], chromothripsis[30] and chromosomal instability[31]. Large chromosomal alterations can be envisioned as macro-evolutionary events and in some circumstances probably represent a point of no return in the development of resistance, illustrating the importance of early therapeutic intervention.

Together with ecosystem-selective pressures, mutational processes lead to parallel and convergent evolution, as well as spatial segregation of clones in primary and metastatic sites[32]. These pressures include exogenous exposures, internal environmental dynamics and cancer therapies themselves. The effects of selective therapeutic pressure have been well characterized and range from the disappearance of targeted cellular clones, to the acquisition of new resistance mutations, to adaptive responses in signalling and epigenetics and, finally, a complete change in tumour phenotype[33]. In some cases, the effects of therapies (mostly chemotherapy) can be profound and equivalent to inducing a state of genomic instability. For example, in low-grade gliomas, chemotherapy with temozolomide can result in hypermutated tumours at recurrence and in some cases bring about the transformation of tumours to highly aggressive glioblastoma multiforme[34]. Similarly, clonal haematopoiesis—as evidenced by recurrent somatic mutations in leukaemia-associated genes in haematopoietic stem cells—has been associated with prior exposure to radiotherapy and chemotherapy and carries an increased risk of developing leukaemia[35]. These observations should prompt us to weigh carefully the potential unintended consequences of administering chemotherapy or radiotherapy.

A critical clinical issue is how tumour heterogeneity is measured. Currently, heterogeneity is evaluated by genomic sequencing of either archived tumour samples at diagnosis or a subsequent biopsied tumour sample at recurrence. This approach—despite its usefulness in some cases for therapy selection[36]—has serious limitations, as it is unlikely to accurately capture tumour heterogeneity, with obvious implications for cancer therapy. For example, targeting an 'actionable' driver mutation may only prove effective if the mutation is truncal (that is, clonal and present in most subclones and regions of the tumour over its lifetime)[37]. In other cases, finding a given mutation may not be a guarantee of the mutation being clonal, and conversely, the paucity of a mutation does not guarantee that it is incidental. Indeed, subclonal driver mutations in *ESR1*[38] and in genes of the PI3K pathway[39] are sufficient to drive resistance to targeted therapies. In this setting, we propose that a catalogue of 'clonality' of driver mutations may be informative.

Physical barriers

Cancer cells can create spatial gradients within tumours that prevent adequate blood flow, thereby creating a pro-tumorigenic hypoxic environment and decreasing the effective exposure of a tumour to drugs. Although attenuating blood flow to tumours is one known mechanism of action of anti-angiogenic agents, this is probably not the complete story. Alternative evidence suggests that anti-angiogenic agents may also normalize vascular structure and function[40], facilitating the delivery of systemic agents such as chemotherapy or even targeted therapy[41]. Recently, the combination of anti-angiogenic tyrosine kinase inhibitors and anti-PD-1/PD-L1 antibodies has also demonstrated what appears to be synergistic activity[42,43], although the precise mechanism underlying this clinical observation is uncertain.

Cancer cells may colonize and proliferate in 'sanctuary sites', or anatomical spaces in which systemically administered drugs do not reach therapeutic concentrations. The prototypical example of this is the central nervous system (CNS) and the physical boundary imposed by the blood–brain barrier. Additional sanctuary sites include the peritoneum, which can be addressed by intraperitoneal chemotherapy, and the testes, which has led to the administration of prophylactic radiation in children with acute lymphoblastic leukaemia.

Of these sanctuary sites, the CNS represents perhaps the highest unmet medical need. Some tumour types such as lung, HER2-positive breast, melanoma and kidney cancers have a particularly high level of CNS tropism. A variety of approaches have attempted to address this daunting clinical challenge, including improved radiotherapy techniques that allow a more selective targeting of tumours in the brain[44], and targeted therapies that penetrate the blood–brain barrier[45]. Early investigations into the combination of immune checkpoint inhibitors and stereotactic radiosurgery have shown promise in inducing rapid and complete response of each lesion compared with stereotactic radiosurgery alone[46]. These data suggest that attacking a macroscopic brain metastasis with a combination of a local therapy and systemic checkpoint inhibition may be a promising approach for controlling CNS metastases. Ultimately, the solution to CNS invasion is not obvious but we are beginning to develop a deeper understanding of the underlying mechanisms that lead to migration across the blood–brain barrier, as well as CNS tropism and growth[47–49], which may translate into therapeutic approaches.

Immune system and tumour microenvironment

The tumour microenvironment—the surrounding space composed of immune cells, stroma and vasculature—may mediate resistance by several mechanisms, including preventing immune clearance of tumour cells, hindering drug absorption and stimulating paracrine growth factors to signal cancer cell growth[50].

The importance of immune evasion by tumours is underscored by the success of checkpoint blockade immunotherapy[14], which has led to sustained long-term control of disease in advanced melanoma, renal cell carcinoma, non-small-cell lung cancer (NSCLC), urothelial cancers and microsatellite-unstable cancers, among others. Some immunotherapy-resistant tumours have a low mutational burden, which leads to a paucity of neoantigens that are available for presentation and ultimately prevents recognition[51,52]. Some mechanisms of resistance to checkpoint blockade have been elucidated, including the loss of β2-microglobulin (which impairs tumoral antigen presentation) and *JAK1* or *JAK2* mutations (which render tumour cells insensitive to interferon gamma)[53].

Immunosuppressive cancer microenvironments—so-called 'immune deserts'—are now recognized as a major impediment to checkpoint inhibitors, owing to the presence of regulatory T cells, myeloid-derived suppressor cells, tumour-associated macrophages, cytokines and

chemokines—all of which can inhibit immune-mediated anti-tumour effects[50]. This has led to the investigation of a range of techniques to turn immunologically 'cold' tumours into 'hot' ones by recruiting immune effectors. These techniques combine checkpoint blockade with anti-angiogenic agents[42,43], targeted therapies[54], metabolic modulators, oncolytic viruses, epigenetic therapies and other checkpoints.

Undruggable genomic drivers

Despite a growing number of successes in efforts to target oncogenic driver mutations, some of the most formidable oncogenes and tumour suppressor genes remain undruggable, including *MYC*, *RAS* and *TP53*. Several approaches are being explored to address these targets, including miniproteins that prevent MYC dimerization[55], allele-specific inhibitors that trap and inactivate mutant KRAS(G12C)[56] and small molecules that covalently bind to p53 to restore its normal (wild-type) function[57].

Other oncogenic drivers, such as class 2 mutations in *BRAF* (which result in mutant BRAF proteins that signal as RAS-independent constitutive dimers[58]), are only partially inhibited by existing allosteric MEK inhibitors[59], owing to an inadequate therapeutic index. New classes of RAF inhibitors that inhibit the formation of RAF homodimers and heterodimers may address these alterations. Furthermore, target indifference—in which the effects of targeting an oncogenic driver are attenuated by downstream or parallel alterations in the pathway—can drive resistance. This is exemplified by resistance to anti-EGFR therapies in colon cancer, which can be mediated by downstream KRAS- or NRAS-activating mutations[60].

Selective therapeutic pressure

Cancer therapies are powerful inducers of changes in both the tumour and its ecosystem. Conventional chemotherapy and radiotherapy enhance genomic instability, with massive and widespread effects on surviving cells and non-cancer cells[28,61], and can also induce immune responses in the host that attenuate anti-tumour responses[62].

Under targeted therapies, changes are subtler and may be divided into early adaptive responses or—after prolonged exposures—acquired resistance. Adaptive responses can occur so rapidly that no response is ever clinically apparent, and may be responsible for short-lived durations of clinical response. Adaptive mechanisms are often the result of non-genetic relief of negative feedback of signalling pathways and/or epigenetic modulation, which causes the activation of parallel pathways, or reactivation of the initial pathway[63,64]. For example, *BRAF*-mutant colorectal cancers are insensitive to BRAF inhibitors, owing to reactivation of upstream receptor tyrosine kinases including EGFR[65,66], but *BRAF*-mutant melanomas express only low levels of EGFR and are therefore not subject to this relief of negative feedback.

In acquired resistance, in which prolonged clinical response is followed by tumour regrowth, other mechanisms are involved that include the emergence of new activating mutations on the target itself, pathway alterations or even histological changes. In monogenic tumours, resistance to tyrosine kinase inhibitors (TKIs) is frequently driven by gatekeeper mutations[67] and other driver mutations, which maintain oncogene dependence[68] by preventing access to the site where ATP-competitive TKIs bind. Other acquired causes of TKI resistance include amplification of the gene and splice variants. Drug-discovery efforts have resulted in newer TKIs that can overcome on-target acquired resistance. Some of these drugs avoid the steric hindrance that is mediated by mutations in the kinase domain (such as the anti-BCR-ABL agent ponatinib[69]), bind more potently to the target (as exemplified by the anti-ALK agent alectinib[70]) and may even exhibit selectivity for mutant kinases by binding irreversibly to the mutated domains (such as osimertinib[71]). Characteristic mutations that are associated with acquired resistance can also develop in the ligand-binding domains of nuclear hormone receptors, resulting in ligand-independent transcription[72].

Another mode of acquired resistance to kinase inhibition is the amplification of an upstream gene, which can bring about resistance to therapy that targets a downstream molecule; this is exemplified by *MET* amplification driving resistance to EGFR inhibitors[73]. Tumour suppressor mutations can also drive resistance to targeted therapies, including mutations in *PTEN*—which drive PI3Kβ signalling in response to PI3Kα inhibitors[74]—and reversion mutations in *BRCA1* or *BRCA2* in response to PARP inhibitors[75].

Finally, phenotypic changes may occur that result in the evolution of treated tumours into new histological types. For example, transformation to aggressive clinical neuroendocrine phenotypes has been noted in prostate tumours that are initially responsive to antiandrogens, and in *EGFR*-mutant NSCLC that is initially responsive to TKIs. These tumours are treated, like newly diagnosed small-cell cancers, with platinum and etoposide chemotherapy, albeit with limited clinical benefit. Genomic studies have revealed that these neuroendocrine prostate and lung tumours that are induced by treatment acquire the loss of *RB1* and *TP53* and become resistant to antiandrogens[76] or EGFR TKIs[77]. Investigations into the mechanisms of changes in cell plasticity have implicated the SOX2 transcription factor, which is upregulated in enzalutamide-resistant prostate cancer[76]. We speculate that additional transcriptional and epigenetic mechanisms drive lineage plasticity in cancers that are responsive to targeted therapies, leading to target independence.

Overcoming resistance

Although any single biological determinant of resistance can contribute to treatment becoming refractory, these factors frequently coexist in a cancer, arising in a time- and therapy-dependent manner. In the next sections we lay out four general solutions to drug resistance—earlier detection of disease; deepened responses; therapeutic monitoring and adaptive interventions; and exploitation of cancer dependencies—and illustrate how these interventions may increase the overall probability of cure (Fig. 2a). We also discuss how new diagnostic and therapeutic technologies are being used to disrupt the emergence of drug resistance.

Earlier detection

Treatment of cancer when tumour burden and clonal diversity are low is perhaps the most obvious strategy to prevent drug resistance. Implementing this approach has two critical components: first, early disease detection; and second, treatment with the most effective upfront therapy to maximize tumour eradication (Fig. 2b). The latter concept is the basis for adjuvant therapy and has improved patient survival for many types of tumour. The most extreme realization of this approach would be cancer interception[78]—the detection of premalignant lesions followed by mechanistically based interventions to prevent the formation of a cancer. We will examine both components below.

Cancer screening, or efforts to detect pre-symptomatic cancer, is currently established in breast, cervical, colorectal and lung cancers and has generally led to improvements in disease-specific survival[79]. However, the tests that are currently available provide low sensitivity and specificity, often detect resistant and incurable disease and can also be tedious and invasive, which leads to poor compliance. A case in point is mammography, which even when optimally used in large populations of women who are at average risk for developing breast cancer reduces breast-cancer-specific mortality by only 20%[80]. This modest benefit is achieved at the expense of a high false-positive rate: over a 10-year period, almost a third of women screened will have had a benign biopsy after a positive radiographic finding[81]. Similarly, prostate-specific antigen (PSA) screening detects mostly low-grade disease but does not decrease mortality[82].

Over the last two decades, it has become possible to detect DNA in the blood that originates from the fetus in pregnant women[83,84] and from tumours in patients with cancer[85]. Circulating tumour DNA (ctDNA) testing offers many potential advantages, including non-invasive,

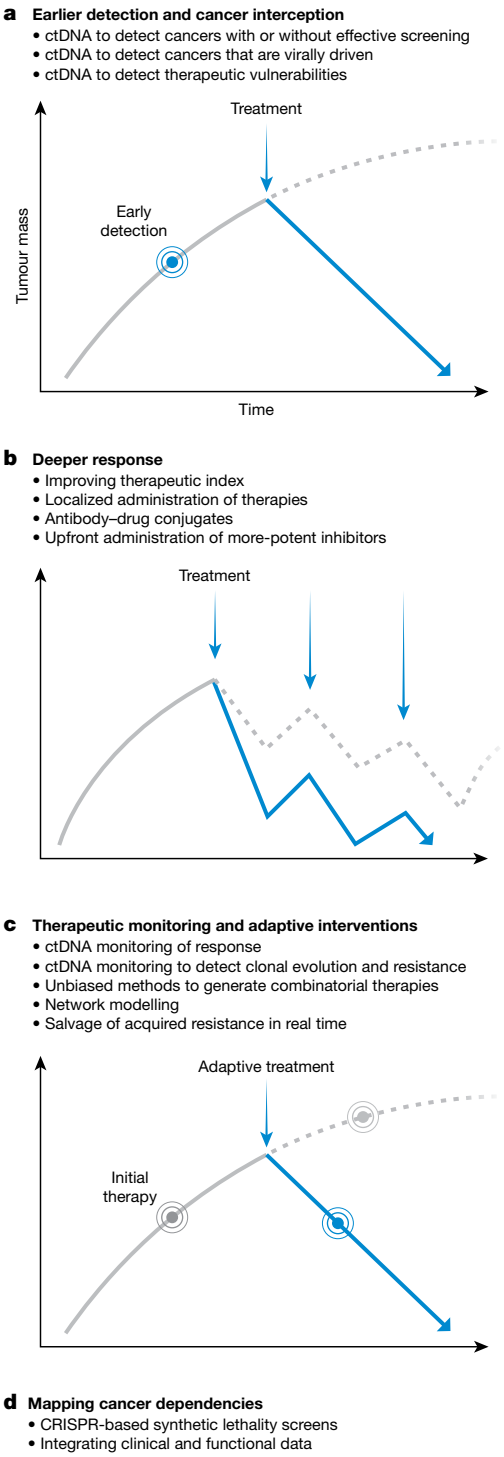

Fig. 2 | Proposed solutions to the problem of drug resistance in cancer. **a**–**d**, Proposed solutions. Many technologies—including plasma-based tumour diagnostics, more-potent pharmacological strategies, computational and systems biology modelling and genetic screening—may help to prevent cancer drug resistance through interceding earlier; enhancing therapeutic efficacy; ascertaining disease burden and changing therapies; and beginning to detail the behaviour of cancer cells under therapy. Early detection and treatment of cancer (**a**), deeper therapeutic responses (**b**), therapeutic monitoring with adaptive interventions (**c**) and mapping cancer dependencies (**d**) can alter the natural history of cancer or its initial therapeutic trajectory to increase the probability of cure (solid blue lines). Grey solid lines show the trajectory before the indicated intervention; grey dashed lines show the trajectory if the indicated intervention were not performed (**a**, **c**) or performed less effectively (**b**).

dynamic and global detection of cancer and monitoring of clonal evolution[86]. The initial finding that ctDNA can be isolated from plasma[87–89]—coupled with methodological developments such as digital PCR[90] to quantify mutant DNA, bead-based emulsions[91] to enhance scalability and, most recently, next-generation sequencing using unique molecular barcoding techniques to correct errors[92,93]—has led to intensive research efforts to develop ctDNA-based 'liquid biopsies' for non-invasive detection[94]. We discuss ctDNA as a tool for cancer screening and surveillance in this section, and examine its role in response and resistance to therapy in later sections.

So far, ctDNA testing has primarily focused on the detection of actionable genomic alterations, including single nucleotide variants (SNVs), copy-number alterations and structural variants. However, for ctDNA to form the basis of an effective screening tool to identify cancer before it metastasizes, it must be able to detect a low burden of disease. Several studies have demonstrated that in early-stage disease, SNV-based ctDNA assays with analytical thresholds that are as low as 0.1% will miss an appreciable proportion of cancers[95], although precise rates vary by cancer type[96–98]. These limitations are not easily overcome by increasing sequencing intensity or error-correction techniques, as the lower limit of SNV detection can be based on the total number of molecular templates available for sequencing—which itself is proportional to tumour burden. Several strategies to improve the ctDNA detection of low disease burden have recently emerged (Box 1). Another challenge for ctDNA detection is the delineation of the tumour tissue of origin. Some new strategies to overcome this hurdle capitalize on epigenetic modifications, combine multimodal methods to distinguish malignant from mutation-based non-malignant processes or focus on detecting specific DNA sequences (Box 1).

Noting these issues, we propose that as a single screening test, ctDNA may initially be used in select subtypes of monogenic cancer, especially those with high rates of cell turnover or in high-risk patient populations (for example, patients with germline *BRCA* mutations). In the case of NSCLC, computed tomography (CT)-based screening is effective in identifying small stage 1 tumours, whereas ctDNA is more effective for larger tumours[95]. Similarly, other cancers may require combined or risk-stratified screening modalities of conventional imaging or laboratory diagnostics (for example, mammography, PSA testing) with ctDNA. To maximize the benefits of early detection, these strategies must be coupled with a definitive therapy to prevent recurrence. Ultimately, population-based screening studies will be required to formally prove the efficacy of ctDNA screening in improving clinical outcomes.

Achieving deeper responses

As the persistence of even a few residual clones inevitably leads to tumour regrowth, the eradication of tumours is imperative (Fig. 2b). Strategies that can be used to deepen responses vary depending on the type of therapy but several guiding principles apply across therapeutic modalities, including optimizing dose, schedule and combination partners.

For chemotherapy, the ceiling for efficacy is often dictated not by a plateau in the dose–response effect, but rather by toxicity or therapeutic index. Nanoparticle platforms[99]—including liposomal or albumin-bound formulations—may mitigate chemotoxicity, but outside of selected cases of leukaemia[100] they have generally not proven superior to the naked chemotherapeutic. In select haematological cancers and germ cell tumours, dose thresholds that are mandated by the limits of bone marrow tolerance have been overcome through the rescuing of marrow function after high-dose chemotherapy by reinfusion of autologous haematopoietic stem cells[101]. However, this approach has been notably unsuccessful in solid tumours[102], suggesting that dose intensification alone will not always deepen responses—possibly owing to alternative mechanisms of promoting tumorigenesis[62]. Optimizing chemosensitivity has long been a goal of cancer therapy; however, ex vivo assays have been largely unsuccessful and patient-derived

Box 1

Emerging methods to improve ctDNA-based screening of cancer

Detection of low burden of disease

Increasing input DNA through phlebotomy of larger volumes of blood[160].

Obtaining non-plasma tumour DNA (for example, stool[161], Papanicolaou smear[162]).

Enriching for ctDNA-sized fragments[163].

Characterizing the tissue of origin

Detecting ctDNA methylation[164].

Assessing the footprints of nucleosome binding[165].

Combining SNVs with epigenomic assays[166] (to distinguish mutations from benign lesions or clonal haematopoiesis[166]).

Distinguishing cancers that are virally driven (for example, EBV-driven nasopharyngeal carcinoma[167]).

Evaluating oncogenic structural variants (for example, translocations[168], amplifications[169]).

xenografts are costly, time-consuming and have not scaled to meet clinical demand. Newer approaches to determine chemosensitivity in vivo include implantable microdevices for real-time multiplex drug testing[103], which could potentially increase the predictive value of chemotherapy benefit.

Another strategy to deepen therapeutic responses is the localized administration of chemotherapy through the liver vasculature, albeit with partial success[104]. A more elegant approach to deliver higher doses of chemotherapy, unconstrained by location or vascular supply, has been enabled by antibody–drug conjugates (ADCs), which consist of recombinant monoclonal antibodies that are covalently bound to cytotoxic agents through synthetic linkers[105]. ADCs enable the delivery of potent cytotoxic payloads directly to tumour cells, as the antibodies are engineered to bind with high affinity to antigens that are preferentially expressed on tumour cells.

Rather than simply increasing local amounts of a less-active chemotherapy, the net effect is to deepen response by localized delivery of a dose of chemotherapy that would be far too toxic if administered alone. Although we acknowledge that ADCs have so far not fully delivered on their initial promise to improve therapeutic index—despite the approval of several of these agents[105]—we believe that with modern ADC chemistry this is beginning to change. This is perhaps best exemplified by the high degree of durable efficacy that is observed with the latest generation of HER2-targeted ADC, DS-8201a, in so-called HER2-low (that is, defined by amplification of HER2 that is less than that necessary for response to current anti-HER2 therapies) breast cancer[106]. DS-8201a demonstrates the ability of a modern ADC to effectively deliver chemotherapy that is based on low levels of expression of a target protein, even when the target protein is not itself an oncogene for that tumour type. We anticipate that further engineering—such as optimizing the ratio of drug to antibody, or varying payloads to include immunomodulatory molecules—may improve the efficacy of ADCs.

With checkpoint blockade immunotherapy, the depth and durability of the response that is elicited have brought about a de facto cure for a fraction of patients with metastatic cancer. Because inhibition of CTLA4 primes and activates T cells at an earlier stage than inhibition of PD-1/PD-L1 (which drives recognition of cancer cells by T cells[14]), a combined anti-CTLA4 and anti-PD-1/PD-L1 blockade can exert a synergistic benefit and elicits a multicellular immune memory. This is distinct from combination targeted therapies, which almost always target multiple intracellular signalling factors and operate on a faster timescale. The notion that immune memory cannot be distilled to a single alteration may explain why PD-L1 is not a universal marker of response to checkpoint blockade across tumour histologies (for example, PD-L1-negative melanomas respond to anti-PD-L1 blockade[107]), and why global measures such as mismatch repair deficiencies in germline or somatic cells[108] or tumour mutational burden[109] can also be used to predict response to checkpoint blockade.

Thus, the nature of durable responses to immunotherapy may be due to the exertion of both on-target (cancer cell–T cell) and off-target (antigen-presenting cell–T cell) effects that contribute to a 'bystander' killing effect on neighbouring cells. Targeted therapies also cause bystander killing, as some targets (for example, the oestrogen receptor) need only be present on a subset of cells for the therapy to eradicate a population of tumour cells. A mechanistic understanding of immunological and non-immunological bystander effects may be relevant for deepening responses to targeted therapies.

Strategies to achieve deeper responses will necessarily be different with agents that target driver oncogenes. We divide targeted therapies into two classes on the basis of therapeutic index: the first (for example, HER2, ALK, NTRK, BRAF, mutant-selective EGFR) with high therapeutic indices and consequently high response rates; and the second (for example, anti-PI3K, MEK, FLT3, mTOR) with low therapeutic indices and lower response rates, owing to either off-target inhibition of other closely related mutant proteins or on-target inhibition of the wild-type protein (which can cause marked side effects as a result of the critical role of the protein in normal homeostasis).

In the case of drugs with a high therapeutic index, combination therapy with non-overlapping mechanisms of action, or more potent derivatives, may deepen or prolong responses. For monoclonal antibodies that target receptor tyrosine kinases, there is no additional dose effect once full target saturation has been achieved. Consistent with this, delivering higher doses of individual anti-HER2 monoclonal antibodies in $HER2^+$ breast cancer does not result in additional benefit[110]. Simultaneously, the safety of naked antibodies affords the opportunity to use full doses of combination agents. In $HER2^+$ breast cancer, the combination of synergistic anti-HER2 antibodies trastuzumab and pertuzumab has resulted in a marked improvement in survival[111]. Whereas most high-therapeutic-index drugs have such a high index because the kinase target has a limited role in normal biological function, other agents with a range of therapeutic indices can achieve efficacy because of their selectivity for mutant over wild-type kinases. We believe that this represents a particularly promising approach to drug development and may even allow targeting of previously intractable alterations such as KRAS(G12C)[56].

For small-molecule inhibitors with a high therapeutic index, multiple agents of differing potency or robustness to resistance mechanisms are available, and upfront application of the best inhibitor or combination appears to result in improved outcomes compared to sequential use. For example, treatment with the more-potent EGFR-mutant-selective inhibitor osimertinib, or the ALK inhibitor alectinib, prolongs progression-free survival in NSCLC compared to first-generation EGFR or ALK inhibitors, respectively[112,113]. In chronic myeloid leukaemia, dual targeting of ABL by a combination of catalytic and allosteric inhibitors drives a potent response that is sustained even when treatment is discontinued[114]. Collectively, these studies demonstrate that using more-potent targeted therapies as a first-line treatment, which maintain activity in the presence of acquired resistant mutations or prevent their appearance altogether, may prolong survival compared to introducing these agents at the time of initial resistance. For example, patients with germline *BRCA*-mutant ovarian cancer who show an apparent partial or complete response after platinum-based chemotherapy can be driven into an even deeper response with two years of maintenance olaparib[115]. The resulting efficacy data, which demonstrate a remission rate of 60% at three years after the start of PARP inhibitor therapy, suggest that this

approach is increasing the fraction of patients that are cured of their disease. By contrast, PARP maintenance therapy after the first relapse of ovarian cancer in an similar patient population prolongs progression-free survival but is not curative[116]. Together, these data suggest that early, aggressive interventions using the most-potent agents (or combination of agents) available may ultimately have more-dramatic effects on long-term patient outcome than sequentially pretreating patients with different drugs.

On the other hand, drugs with a low therapeutic index can be efficacious as monotherapy, but with substantial side effects. PI3K inhibitors yield low response rates as monotherapy, but when used in combination with anti-endocrine therapy in ER$^+$ *PIK3CA*-mutant breast cancer they improve progression-free survival[117]. However, this comes at the cost of on-target side effects from insulin inhibition, including hyperglycaemia. Improving the depth of response to drugs with a low therapeutic index will require new approaches; for example, combining PI3K inhibitors with a ketogenic diet that blunts adaptive exogenous insulin signalling and resensitizes cells to PI3K inhibitors[118]. A unique example of an agent with a low therapeutic index is the BCL2 inhibitor venetoclax, which lowers the apoptotic threshold of cells and produces very deep responses in chronic lymphocytic leukaemia[119]. However, venetoclax can also lead to dramatic tumour lysis syndrome in patients with a high burden of disease before the onset of therapy; this on-target effect can be successfully managed with careful dose escalation.

Quantifying the depth of response to surgery or systemic therapy with ctDNA may provide opportunities to identify high-risk groups for additional therapeutic intervention, and predictive biomarkers in systemic therapy. After primary breast surgery, detection of minimal residual disease by ctDNA was possible eight months before detection by imaging, creating a lead time in which additional therapies could be tested to improve the depth of response[120]. The detection rate of minimal residual disease after surgery is improved in early-stage NSCLC by tracking five mutations by ctDNA rather than one[121]. In metastatic breast cancer[122] and *BRAF*-mutant colorectal cancer[123], decreased levels of ctDNA correlate better with therapeutic response than standard tumour markers. For immunotherapies, global ctDNA measurements—such as a high tumour mutational burden in the plasma—may correlate with response to checkpoint blockade[124].

Monitoring of response and adaptive interventions

If we assume that our approach to cancer therapy is at best empirical, real-time monitoring of response to therapy would enable earlier modifications in dose, schedules and therapeutic regimens (Fig. 2b). Unfortunately, the conventional way of assessing response is based on measuring changes in tumour diameter using serial radiographic imaging. More recently, functional imaging during therapeutic intervention has enabled a faster and more-precise assessment of response in some tumour types. For example, using positron emission tomography–computed tomography (PET–CT) scan-based functional imaging to assess a patient's interim response to ABVD chemotherapy (that is, a combination of adriamycin, bleomycin, vinblastine and dacarbazine) is now standard in Hodgkin's lymphoma, after which treatment can be escalated or de-escalated accordingly[125].

Acquired resistance is often ascertained by a single biopsy; however, the heterogeneity of resistance can manifest as multiple resistant subclones in multiple metastases[74,126]. In addition to functional-imaging techniques, there is growing evidence that ctDNA-based methods can be a valuable tool for monitoring tumour heterogeneity, evolution and response to therapy. As with HIV, for which viral load is used to monitor response and adherence to therapy, 'tumour load' (as measured by ctDNA) could be used in a similar fashion (Fig. 2b). Real-time monitoring by ctDNA may also provide a time window in which to identify patients who may relapse[127–129], as well as providing information on clonal evolution under therapy and thereby helping to dictate the next therapy at an earlier time point, especially when a dominant resistance mutation is likely to arise[130,131]. Techniques including the barcoding of endogenous DNA[132] may enable tracking of intratumoral heterogeneity. In *EGFR*-mutant NSCLC, detection of the most common resistance mutation, *EGFR*^{T790M}, through ctDNA is now a standard of care for patients whose disease progresses after treatment with first-generation EGFR inhibitors. Moreover, detection of resistance mutations by ctDNA can enable therapy with second-line EGFR agents to begin before clinical resistance manifests (for example, in patients who received first-line erlotinib)—potentially further prolonging survival[133]. Early ctDNA detection of mutant *KRAS* alleles that arise in metastatic colorectal cancers as a result of acquired resistance to anti-EGFR antibody therapies potentially provides a therapeutic imperative to stop anti-EGFR treatments[134].

At present, a more daunting challenge than the emergence of acquired resistance mechanisms is how to address responses that are initially adaptive but that result in outright resistance or short-lived clinical benefit. Robustness, a property that allows a system to function despite external and internal perturbations, is a defining feature of cancer. It is therefore not a surprise that activation of compensatory pathways is a prevalent mechanism of resistance to targeted therapies[64]. These compensatory adaptation processes are rapid and either result in reactivation of the targeted pathway itself (either upstream or downstream) or engage signalling nodes that bypass the oncogenic pathway. Although such compensatory processes are a sobering reality when attempting to target oncogenic drivers, we propose that identifying the 'drivers' that underlie the feedback mechanisms in each case could be therapeutically exploited.

Some oncogene targets—such as *BCR–ABL* in chronic myeloid leukaemia, gene fusions in members of the NTRK family in a variety of tumour types, or mutant *EGFR* in lung cancer—do not result in an adaptive response that is clinically meaningful. Classically, these tumours respond to their respective targeted therapies for prolonged periods of time until mechanisms of acquired resistance eventually take over. On the other side of the spectrum, adaptive responses are the norm when components of key cellular pathways such as the HER2–PI3K–AKT–mTOR and RAS–RAF–MEK–ERK pathways are targeted[135–137]. Similarly, targeting ER in breast cancer and AR in prostate cancer induces rich cellular responses that reduce the effects of internal or external perturbations. If we consider as a whole these targets that elicit powerful adaptive responses, they share some obvious commonalities: in particular, they belong to cellular pathways that are critical for both normal and cancer cells and that are highly regulated by complex feedback processes.

To some extent, these adaptive cancer dependencies can be identified through experimental testing. For example, genes that confer resistance to inhibition of the MAP kinase pathway in *BRAF*^{V600E}-mutant metastatic melanoma have been identified through gain-of-function genetic screens and genomic or transcriptomic sequencing of melanomas that are resistant to therapy. These large datasets of putative genes that confer drug resistance converge on ERK-dependent, ERK-independent and pathway-indifferent states that induce cellular pathway reactivation[138–140]. Similar examples have been observed in ER$^+$ *PIK3CA*-mutant breast cancers, in which inhibition of PI3Kα elicits epigenetic reprogramming and increases ER transcription[141,142].

Resistance to immunotherapy is necessarily different from resistance to targeted therapy. The direct targets of checkpoint blockade (PD-1 and CTLA-4) and their ligands are necessary to raise the threshold for activation of T cells and restrain the function of effector T cells, but are neither necessary nor sufficient for oncogenesis itself or response to checkpoint blockade. Moreover, antigen-presenting cells and T cells are genomically more stable relative to cancer cells. Thus, simple mutation or downregulation of PD-1, PD-L1 or CTLA-4 is not a common compensatory resistance mechanism; although mutation of PD-L1 would theoretically impair the recognition of checkpoint blockade antibodies, it would also impair the normal function of antigen-presenting cells and T cells. Rather, mechanisms that downregulate the presentation

of major histocompatibility complex (MHC) proteins in cancer cells—such as mutation of *B2M*, *JAK1* or *JAK2*—predominate among the known mechanisms of resistance to immunotherapy[53]. Adaptive responses that may hypothetically increase sensitivity to checkpoint blockade, such as DNA damage that is induced by chemotherapy, are under active clinical investigation. Serial multiplex testing of both the tumour and other cell types of the tumour microenvironment could enable the elucidation of adaptive responses to immunotherapy.

In addition to the type of experimental designs that we have described, adaptive responses can also be inferred from less-biased technologies that make few assumptions about the system, including network modelling. Logic-based models in which proteins are denoted as nodes can reconstruct cell signalling pathways[143], and discrete dynamic models can be constructed that assess the node read-outs of inhibitory and activating states over time. The models can undergo computational simulations to determine the effects of an inhibitor and the relative fitness of different mechanisms of resistance in modulating apoptosis and proliferation. As an example, dynamic modelling of PI3K inhibition in ER^+ breast cancer predicts that the combination of CDK4/6 inhibition together with PI3K inhibition can resensitize cells to PI3K inhibition[144].

Another way to improve drug response would be to adapt the process of drug development. The traditional approach to developing drugs that target resistant mutations was to test the parent drug clinically, identify patients who develop resistance, molecularly characterize resistant tissue, model newly identified mutations preclinically and, finally, design new drugs for clinical testing. Unfortunately, this process takes many years, meaning that patients who experience a new first-in-class targeted therapy rarely live to receive a next-in-class agent. However, we are finally beginning to see examples of this bottleneck being overcome. Studies have shown that the selective TRK inhibitor larotrectinib is highly efficacious in an age- and tumour-agnostic manner in *TRK*-fusion-positive cancers[145]. Even before any acquired resistance mutations were detected in patients, preclinical and in silico modelling accurately predicted which mutations of the kinase domain of *TRK* would drive resistance to larotrectinib, and enabled the development of mutant-selective inhibitors. For those initial patients who received larotrectinib and developed acquired resistance, it was possible to move a next-generation TRK inhibitor into the clinic in a timeframe that enabled salvage of the therapeutic response[146].

Mapping cancer dependencies

Some cancer dependencies have been discovered through classic methods of drug screening in cell lines—as in the case of the combination of ER and CDK4/6 antagonists in the treatment of ER^+ breast cancer, which results in a synergistic control of disease compared to anti-ER therapy alone[147]. Exploring synthetic lethality, in which the concurrent perturbation of two genes leads to cell death, is another approach to identifying therapeutic vulnerabilities to a known driver mutation. The classical example of this phenomenon is PARP inhibition in *BRCA*-mutant tumours in which both DNA-repair genes are in parallel pathways[148]; however, this concept has now been expanded beyond tumour suppressors to discover new target genes that are synthetically lethal to selective oncogene inhibition or other specific drug therapy[149].

Deciphering resistance to immunotherapy requires an understanding of the interplay between mechanisms that are intrinsic to cancer cells and extrinsic cellular and humoral factors, and these intrinsic and extrinsic components are likely to be different when comparing resistance to checkpoint blockade, adoptive cellular therapy, bispecific T cell engagers and cancer vaccines. Using small hairpin RNA (shRNA) to disrupt genes and performing high-throughput screening for loss of function can identify genes whose deletions cause resistance or enhance sensitivity to a specific driver or drug. This approach is limited, as gene knockdown may not be complete and off-target effects are common[150]. However, these problems can be overcome by CRISPR–Cas9 technology, which yields fewer off-target effects and increased efficiency given that the integration of a single-guide RNA can cleave both copies of a target DNA molecule[151,152]. One of the first genome-wide CRISPR-based knockout screens was used to identify potential mediators of resistance to RAF inhibition in *BRAF*-mutant melanoma that had not previously been defined by shRNA-based screens[152]. This has spurred a flurry of loss-of-function genome-wide CRISPR screens that have uncovered new genes associated with sensitivity and resistance[153–155] to immune checkpoint blockade.

Integrating large clinico-genomic datasets with the outputs of synthetic lethality screening and computational modelling might one day facilitate a real-time reference guide of cancer dependencies to be produced for a diverse range of cancer types, with the ultimate goal of using predictive analytics to select combination drug candidates in a rational manner. For example, pan-cancer loss-of-function shRNA screens with deep coverage, analysed using subtraction methods to account for off-target effects, have revealed novel synthetic lethalities—including those that affect subunits in the SWI-SNF (switch/sucrose non-fermentable) chromatin remodelling complex, PRC2 repressor complex and Mediator complex[156,157]—which suggests that cancers driven by 'undruggable' targets may be targeted by drugging other enzymatic components within the same protein complex. Analysing these changes across therapy-naive and drug-resistant lines of cancer cells may reveal consensus dependencies that could eventually be exploited therapeutically.

In monogenic diseases such as chronic myeloid leukaemia, this level of analytics may not be required as the path to therapy is well defined. However, in most other cancers, which have a few dominant drivers but also a large number of known and unknown resistance mechanisms, the best treatments and orders of treatments to overcome drug resistance have not been established. In these cases, uncovering highly efficient combinations of therapies through a number of strategies including simultaneous co-targeting of multiple truncal alterations; targeting histology-specific synthetic lethal vulnerabilities; or identifying higher-order synthetic lethal interactions of perturbations that affect more than two genes may help to define new drug combination strategies[158].

Conclusion

Resistance to therapy continues to be the biggest challenge in cancer today. There are as many underlying mechanisms of resistance as there are patients with cancer, because each tumour has its own defining set of characteristics that dictates tumour progression and that can eventually lead to death. Solving the resistance problem would therefore seem to be an unattainable goal. Here, we have proposed the creation of a framework that dissects and partitions resistance into its biological determinants. This enables the different problems to be tackled as separate working units at first, and then considered in a global manner.

Combining an assessment of the physical properties of the tumour (tumour burden, growth rate and localization) together with a deep analysis of tumour drivers and druggability, dependencies and vulnerabilities, early detection and precise monitoring and, finally, powerful analytics based on patient databases will no doubt be a challenging but formative approach to fighting the resistance of cancer to therapy. This combined knowledge should enable the development of tools that are capable of informing real-time clinical decisions, and which continuously improve through a feedback process that is reiterated by observed phenotypic outcomes. We recognize that many of the solutions proposed here would be costly at first, and further tax those systems of healthcare that are already strained[159]. These are important issues, and addressing them will require fundamental changes to economic models of oncology care.

Despite the caveats, it does finally seem possible—at least conceptually—to outline a roadmap for tackling the problem of resistance by using our understanding of its biological building blocks. Although we still need to fully understand key tumour drivers and their regulation

(such as epigenetic and metabolic factors), cancer resistance has increasingly begun to resemble an engineering problem. We envision an amalgamation of engineering and cancer biology that will advance the field in unprecedented ways. The last example of a leading cause of death being eradicated by medicine was in the field of infectious diseases. As a next progressive step in the history of medicine, perhaps it is a reachable goal within our lifetimes to deliver cancer's coup de grâce.

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