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Perspective

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The coming decade in precision oncology: six riddles

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Abstract

High-throughput methods to investigate tumour omic landscapes have quickly catapulted cancer specialists into the precision oncology era. The singular lesson of precision oncology might be that, for it to be precise, treatment must be personalized, as each cancer's complex molecular and immune landscape differs from patient to patient. Transformative therapies include those that are targeted at the sequelae of molecular abnormalities or at immune mechanisms, and, increasingly, pathways previously thought to be undruggable have become druggable. Critical to applying precision medicine is the concept that the right combination of drugs must be chosen for each patient and used at the right stage of the disease. Multiple puzzles remain that complicate therapy choice, including evidence that deleterious mutations are common in normal tissues and non-malignant conditions. The host's role is also likely to be key in determining treatment response, especially for immunotherapy. Indeed, maximizing the impact of immunotherapy will require omic analyses to match the right immune-targeted drugs to the individualized patient and tumour setting. In this Perspective, we discuss six key riddles that must be solved to optimize the application of precision oncology to otherwise lethal malignancies.

Sections

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Introduction

"'Poirot,' I said. 'I have been thinking.' 'An admirable exercise my friend. Continue it.'"
Agatha Christie (Peril at End House)[1]

Paul Ehrlich's concept of a magic bullet for microorganisms[2,3], conceived roughly a century ago, is analogous to modern oncology's greatest ambition: targeting a tumour — and potentially its surrounding growth-promoting microenvironment — without causing harm to normal tissue. The past three decades have yielded technological advances likely to be the cornerstone for achieving this long-awaited goal; namely, next-generation sequencing (NGS) and advances in computing power and related bioinformatic algorithms. Whereas the first sequencing of the human genome, in 2001 (refs.[4,5]), took approximately 13 years and cost approximately US $2.7 billion, sequencing can now be performed in a matter of hours for a small fraction of this cost[6]. This has allowed the use of sequencing to identify cancer-causing genetic drivers in individual patients and even in different clonal populations in tumours, and the selection of drugs to act upon identified vulnerabilities such as deregulated pathways or proteins in tumour cells or tumour immune cells, which has improved the prognosis of some cancers.

Although we have achieved substantial progress in cancer therapy and have even been able to find 'magic bullets' for a few cancer types by exploiting genomics and precision oncology treatment paradigms, as discussed in riddle 1 — most prominently chronic myeloid leukaemia (CML), a previously lethal malignancy that now has a life expectancy approaching normal[7] — traditional cytoreduction approaches such as chemotherapy, radiotherapy and surgical resection are still heavily relied on as treatments for most cancers. CML treatment might serve as a blueprint for subsequent precision therapies, and it is, therefore, useful to briefly recapitulate the history of this revolutionary therapy[8]. In 1959, David Hungerford and Peter Nowell observed a genetic lesion in cells of patients with leukaemia — an abnormally short chromosome 22 (ref.[9]). Named after the city of its discovery, the Philadelphia chromosome was the first genetic defect linked to a specific cancer type. Roughly a decade later, in 1973, the translocation t(9;22) at the root of this defect was described by Janet Rowley, leading to the identification of the *BCR–ABL* fusion gene (*ABL* is also known as *ABL1*), which results in the abnormal expression of the fusion protein and tyrosine kinase BCR–ABL[10–16]. Despite the identification of a unique mechanism of tumorigenesis, drug development efforts for targeting BCR–ABL were almost cancelled owing to the presumption that targeting a specific kinase domain would affect others and result in toxic effects[17]. Eventually, a few grams of the BCR–ABL inhibitor imatinib were synthesized and released to Brian Druker, who initiated the first clinical trial, in 1998. Only 2.5 years later, the US Food and Drug Administration approved the use of imatinib in patients with CML[14]. By targeting of the aberrant BCR–ABL kinase in CML with imatinib and subsequent generations of BCR–ABL inhibitors (for example, nilotinib, dasatinib and ponatinib) to subvert its activity and prevent the acquisition of secondary mutations[17–20], CML has been transformed from a death sentence to a mostly chronic disease, relatively easily managed over the course of the patient's life[18–21].

The success of a single type of precision targeting agent achieving near-universal durable benefit is mostly unparalleled to this day in solid tumour oncology. Careful consideration of the key ingredients that led to success in CML is crucial to understanding whether CML is fundamentally different from other, mostly solid cancers, for which we have identified underlying molecular drivers. Indeed, the precision targeting of solid cancers has not yielded anywhere near the universal responsiveness seen in CML, and responses are not as durable. In this Perspective, we discuss six riddles that must be solved to optimize the application of precision oncology to solid cancers, using CML as a blueprint for successful precision therapy.

Riddle 1: Is it about time?

'Time is the best killer.'
Agatha Christie

There were three key components to overcoming CML. The first was the discovery of the abnormal *BCR–ABL* gene. The second was the discovery of imatinib, a drug that specifically suppresses the aberrant BCR–ABL kinase and hence targets BCR–ABL-mutant leukaemia cells without killing normal cells that depend on other kinases. These first two components — identifying the molecular driver and administering a matched targeted agent — have been achieved for other cancers with some success, for example with the development and application of EGFR, ALK, RET and NTRK inhibitors[18–21]. Although these inhibitors have been applied to solid cancers with improved outcomes, their success is nowhere near that of imatinib. For example, *ALK* rearrangements are an important driver of lung cancer, yet ALK inhibitors yield response rates of ~55% with a median duration of 7 months in lung cancer[21] — gratifying but a far cry from what is observed with the use of BCR–ABL inhibitors in CML. This observation has led to a common claim that CML is just different and perhaps less complicated than solid cancers[22]. However, we postulate that CML is not fundamentally different from solid malignancies, and that it is the missing third ingredient, timing, that holds the therapeutic key[8,16] (Fig. 1).

Imatinib applied to end-stage CML at the blast transformation or blast crisis stage shows cytogenetic response rates of ~10% with a median survival of about 1 year[23]. The low effectiveness of imatinib at this stage is because clonal evolution has occurred, and, although BCR–ABL remains a driver, additional molecular abnormalities emerge and act as co-drivers. Today, precision solid cancer oncology strives to replicate the CML paradigm by applying targeted therapies to advanced tumours that exhibit multiple co-drivers;[24] however, targeted therapy of solid tumours in the metastatic setting is equivalent to using imatinib during blast crisis CML. Almost all targeted therapies for solid cancers have been administered to patients with advanced metastatic disease who have been heavily pretreated[16], and, although some of these patients respond, responses are often short-lived and most of these individuals do not achieve the near-normal life expectancy seen in imatinib-treated patients with CML. Applying therapy early in the disease course, before complex clonal evolution of the malignancy has occurred, might be crucial to increasing the success of targeted treatment in solid cancers; therefore, analysis of molecular genetic lesions in tumours should be considered as a first-line strategy in all patients with cancer to ensure early and accurate treatment: the right drug or drugs to the right patient at the right time.

On the other hand, it is possible that the therapeutic strategy in CML is different from those targeting other, solid tumours in that it represents the targeting of a gain-of-function mutation in a disease with little other genomic complexity. Most solid tumours involve the co-mutation of multiple drivers, some of which may occur early in tumorigenesis. Further, some of these drivers are loss-of-function mutations that have proved difficult to target with drugs, and ABL inhibitors might have less off-tumour toxicity than some other inhibitors.

Therefore, replicating the success of imatinib may not simply be a case of timing alone. Answering this riddle will require carefully designed clinical trials that further investigate matched targeted drugs early in the disease course of solid tumours.

Riddle 2: When is a deleterious mutation pathogenic?

'One of us in this very room is in fact the murderer.'
Agatha Christie (And Then There Were None)[25]

A critical riddle relates to malignant transformation – the acquisition of somatic mutations that transform healthy cells into cancer cells. The balance of tumour promoters and suppressors ultimately moulds the dynamic process by which cells obtain the characteristics of cancer through a process of clonal selection[26]. This is typified by the adenoma–carcinoma sequence in colorectal cancer, in which the histopathological progression of premalignant adenomas starts with the loss of a single tumour suppressor (APC) and full transformation into a malignant carcinoma occurs following several acquired oncogenic hits[27]. Multiple acquired mutations ultimately lead to the various aberrant features of cancer cells, such as abnormal proliferation, evasion of cell death, angiogenesis and tissue invasion, which independently are topics of intense research and therapeutic targeting[28–30].

A plethora of cancer genomes and transcriptomes have been generated and are being extensively studied[31,32], and the advent of

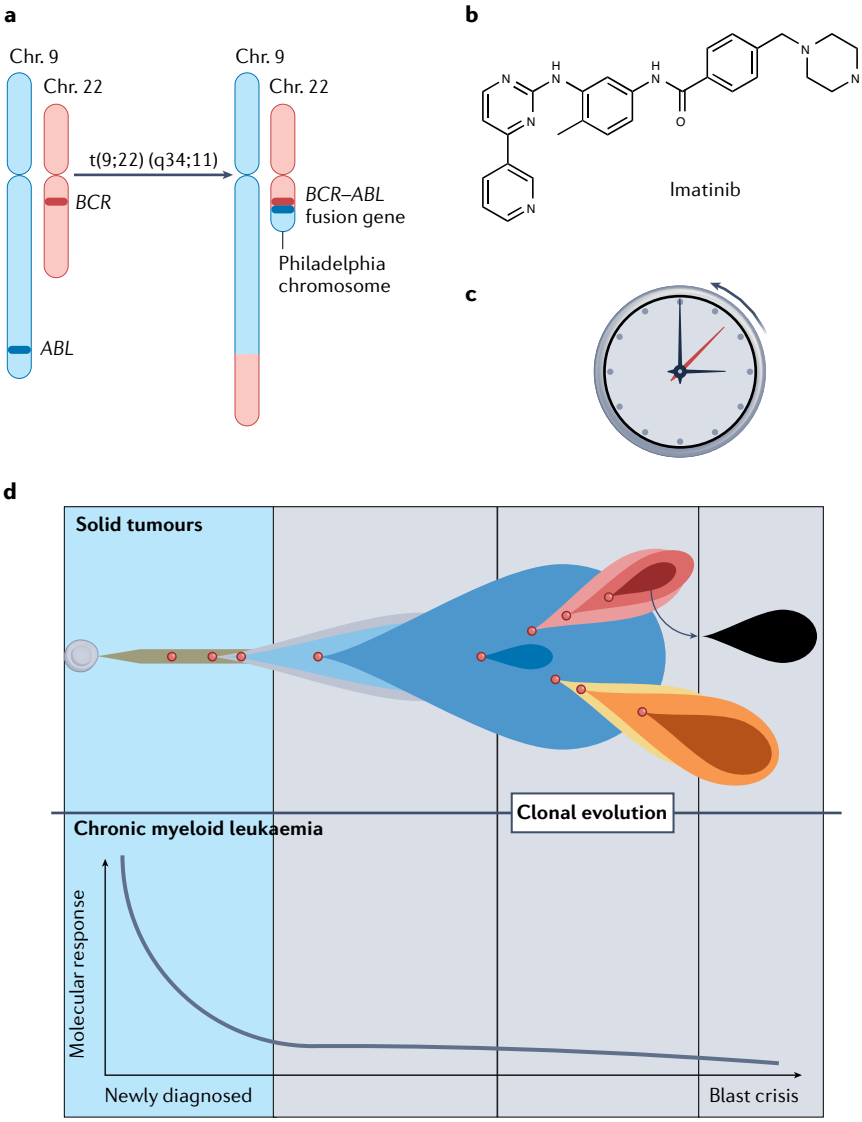

Fig. 1 | Is it about time? The remarkable success of treatment of chronic myeloid leukaemia (CML) could serve as a blueprint for solid cancers. **a**–**c**, The success of CML treatment was characterized by three ingredients: identification of a targetable molecular driver in the form of the *BCR–ABL* fusion gene (part **a**), identifying a treatment (imatinib) to target this molecular driver (part **b**), and early treatment at diagnosis (part **c**). These ingredients should be considered in efforts to target solid cancers. **d**, Treatment of CML with imatinib shows remarkably higher cytogenetic/molecular response rates in patients with newly diagnosed CML than in patients with blast crisis. Blast crisis is likely equivalent to metastatic disease in solid tumours, and thus the time of treatment should be considered in these cancers. Chr., chromosome.

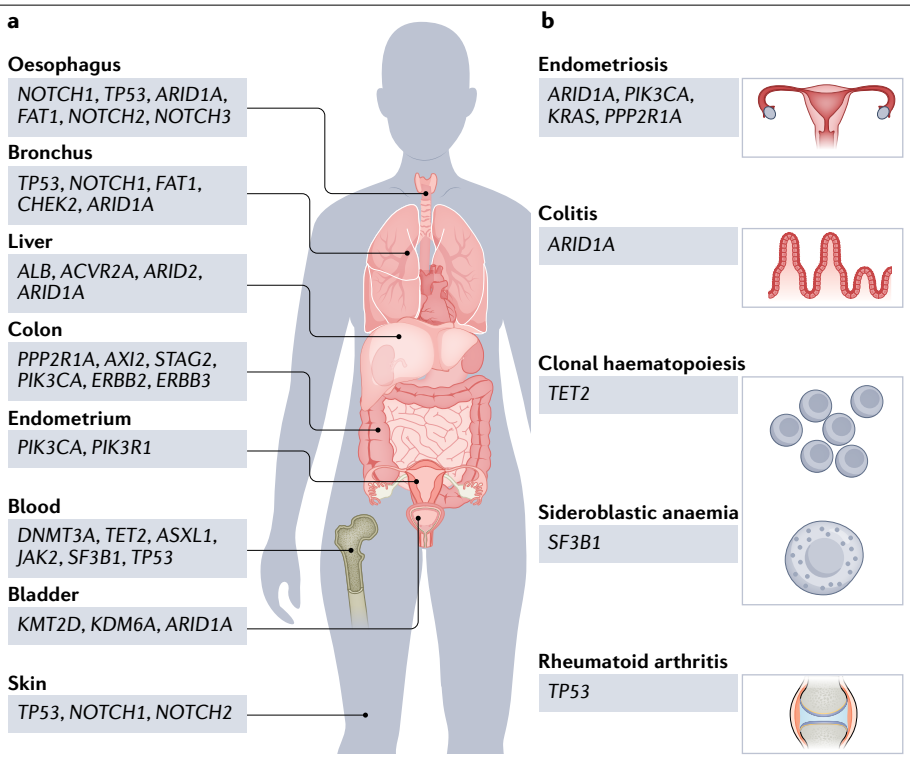

Fig. 2 | When is a deleterious mutation pathogenic? Next-generation sequencing has revealed that deleterious alterations can be found in non-malignant conditions, confounding the assessment of which alterations to target for cancer treatment. **a**, Examples of genes with known oncogenic driver mutations frequently detected in healthy tissues. **b**, Examples of genes with known oncogenic driver mutations that occur in diseases not known to transform into malignant tumours, such as endometriosis[35].

NGS has led to intriguing data concerning the pathogenic role of oncogenic mutations. Unexpectedly, deleterious genomic variants can be seen in non-malignant disease, which confounds the determination of which molecular abnormalities should be targeted when a malignancy is being treated[33,34] (Fig. 2). For example, endometriosis — which harbours little risk of transformation — often bears driver mutations in classic oncogenes including *ARID1A*, *PIK3CA*, *KRAS* and *PPP2R1A*[35]. Mutations in *TP53*, the most common gene implicated in cancer transformation, can frequently be found in the synovium of patients with rheumatoid arthritis, a non-cancerous condition with virtually no risk of malignant evolution[36] (rheumatoid arthritis may be induced by mutant *TP53*-dependent production of interleukin-6 — an inflammatory cytokine that is targeted by approved treatments of rheumatoid arthritis[37,38]).

As high-throughput sequencing is being exploited by oncologists and molecular tumour boards[39–41] (Box 1), the role of deleterious variants in driving cancer must be cautiously confirmed. Emerging data indicate that interpreting the pathogenicity of a mutation goes beyond the determination of whether a mutation results in a deleterious altered function. For example, it is established that deleterious mutations in cancer driver genes are also found in healthy tissue[42]. A whole-genome-sequencing study of healthy colorectal crypts from patients aged between 50 and 60 years revealed approximately 3,000 substitutions and 300 indels (compared with 10,000–20,000 substitutions and 1,000–2,000 indels on average in most colorectal carcinomas)[43]. Furthermore, reverse clonal selection — where the allelic frequency of aberrant genes decreases over the course of malignant progression — appears to occur in some cases; a striking example of this is noted with $BRAF^{V600E}$ mutations. These mutations are a hallmark of about 50% of melanomas and many other cancers and are a pathogenic driver as evidenced by the considerable efficacy of BRAF inhibitors; however, it is puzzling that they are also found in ~80% of benign nevi with negligible risk of cancer transformation[44]. Similarly, whereas *HER2* (also known as *ERBB2*) overexpression in breast cancer is found throughout the benign-to-malignant transition, it is detected more frequently in the benign neoplasm ductal carcinoma in situ (~27–56%) than in invasive breast cancer (~11–20%)[45–47]. Other examples exist as well; in bladder cancer, the frequency of *FGFR3* mutations is inversely correlated with the aggressiveness of the tumour, as grade 1 bladder cancer presents with the highest frequency of *FGFR3* mutations (~60%), whereas the most aggressive, high-grade tumours harbour *FGFR3* mutations in only ~11% of cases[33,48,49].

On a basic scientific level, the preceding observations pose the question as to which molecular events are actually required for a cell to transform into a malignant cancer cell, if not deleterious mutations alone. They could also pose a dilemma for molecular tumour boards, which need to fully comprehend the milieu that cooperates with deleterious mutations to cause cancer. Most importantly, these observations emphasize the complexity of judging genomic alterations when one is evaluating targets for therapy[50–52].

Box 1

Tapping the potential of the molecular tumour board

The success of targeting the BCR–ABL kinase in chronic myeloid leukaemia was made possible through the interaction between basic and clinical scientists who deciphered the molecular aberrations and addressed the clinical challenges posed[134]. Akin to this cooperation, molecular tumour boards (MTBs) are staffed with bioinformaticians, basic scientists, oncologists, geneticists and pathologists, all of whom design a tailored therapeutic approach as the result of a discussion from multiple perspectives, taking clinical, molecular and diagnostic variables into account[130]. There are several hurdles to the success of MTBs in determining the treatment that will give the best outcome. First, MTBs often convene at a point when the cancer is refractory and has evolved in such a way that targeting actionable driver lesions might not derail the oncogenic process. Convening the MTB early in the course of disease might therefore be critical, exploiting the limited dependency of a nascent malignant clone before extensive tumour evolution has occurred. Second, more basic research will be required to understand the pathogenicity of deleterious mutations in their specific tissue context[135]. Therapeutic impact is complicated by the occurrence of competing clones, which cannot always be targeted simultaneously, thereby potentially aggravating interclonal competition, with the overall outcome being unclear. Third, many external factors beyond these tumour-focused considerations shape oncogenesis, including — but not limited to — the intestinal microbiota, the host's genetic background (which can impact immunity, pharmacogenomics and more), sex and age. Lastly, the advent of immune checkpoint inhibition has led to a treatment paradigm that differs from that of single-agent targeted therapies. In single-agent therapies, the presence of multiple mutations can limit the effective treatment of evolved tumours, cancers with high mutational burden responding better to immune checkpoint blockade[136]. MTBs must consider these aspects to achieve the best possible clinical recommendation for the patient affected by a malignancy.

Riddle 3: Do cancer mutations possess tissue tropism?

'Very few of us are what we seem.'
Agatha Christie (The Man in the Mist)[53]

One of the most intriguing features of precision oncology is its tissue-agnostic approach[54]. Several mutations that were found and targeted in specific histologies have been successfully targeted in cancers that originate at other sites. Indeed, the molecular aberrations of a tumour could be as or even more important than its tissue of origin. One obvious example that illustrates this point is the recently approved treatment of a wide array of tumour entities harbouring NTRK gene family fusions with the NTRK inhibitor larotrectinib[54,55]. *BRAF* mutations are often considered[55] an exception to the tissue-agnostic paradigm, as targeting these mutations is an effective strategy for hairy cell leukaemia and melanoma[56,57], whereas *BRAF*-mutated colorectal cancer responds poorly to BRAF inhibitors[58,59]. However, inferior response to BRAF inhibition in $BRAF^{V600E}$ colorectal cancer has been shown to be due to EGFR-mediated reactivation of the MAPK pathway[60,61] (Fig. 3), and BRAF inhibitors are effective in colorectal cancer when co-activated pathways are targeted. This finding ultimately led to the approval of the BRAF inhibitor encorafenib in combination with the EGFR inhibitor cetuximab for BRAF-mutated colorectal cancer[62]. Targeting co-altered signals is also important in the subset of patients who harbour BRAF inhibitor-sensitive cancers such as melanoma but do not respond to BRAF inhibitors or who do respond and develop secondary resistance. Thus, molecular aberrations may be the basis for a new nosology for cancer[55].

While it is becoming apparent that various oncogenic aberrations are shared across cancers and can therefore represent a common focus for pharmacological targeting, it is intriguing why some molecular aberrations are almost unique to specific cancer types. One of the most prominent examples is the *BCR–ABL* translocation, which is a hallmark of CML and rarely found in other cancers, with the exception of Philadelphia-positive acute leukaemia[63]. However, one recent, striking report described beneficial treatment with the BCR–ABL inhibitor imatinib in a patient affected by glioblastoma bearing the BCR–ABL mutation. Overall, the examples above demonstrate that predicting the underlying genomic basis of individual cancers on the basis only of their site of origin is difficult and that universal NGS testing of each tumour is the rational solution to detect unexpected druggable alterations[54].

Riddle 4: Which tumour clone should be targeted?

'They tried to be too clever — and that was their undoing.'
Agatha Christie (The Mysterious Affair at Styles)[64]

One of the most important characteristics of cancer that is not yet therapeutically actionable is clonal evolution. The loss of DNA integrity surveillance mechanisms in this process, such as those perpetrated by mutations of *TP53*, is an important feature of malignant progression. Clonal outgrowth under therapeutic pressure[65] is the main reason for relapse and therapy resistance, thereby representing an important therapeutic challenge. From a precision oncology point of view, this concept is particularly important as actionable targets identified by genomic testing often represent subclonal events that are present in only a fraction of tumour cells. This realization raises questions regarding the therapeutic efficacy that can be expected from targeted therapy as genomic alterations identified in bulk tissue NGS could be irrelevant to some parts of the tumour. The success of precision oncology therefore largely depends on how many clones can be targeted at once while avoiding treatment-related toxic effects. Single-cell sequencing could deliver a more granular picture of clonality before and during therapy than bulk-tissue NGS and might also reflect cell states and lineages in a more detailed way, aspects important to both the response to targeted therapy and the response to checkpoint blockade. Liquid biopsies could also be useful to permit genomic sequencing of DNA shed from multiple metastatic sites.

The consequence that targeting a single clone in a tumour consisting of many clones has for sister clones is poorly understood (Fig. 4). One might imagine that interclonal competition for oxygen or nutrition could be alleviated for sister clones as soon as one clinically targetable clone has been eliminated, which might accelerate the growth of the

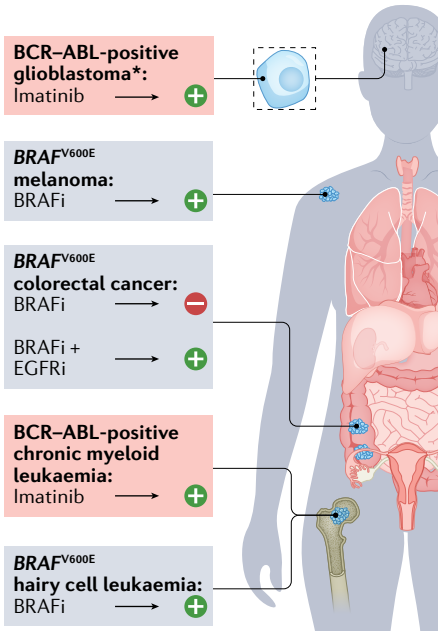

Fig. 3 | Do cancer mutations possess tissue tropism? A key question in cancer is the degree to which certain genomic alterations are present in and impact the growth of specific histologies, and whether tumour-agnostic treatment approaches are effective. For instance, *BRAF*^{V600E} cancers arising from different tissues, including melanoma and hairy cell leukaemia, can be successfully treated with BRAF inhibitors (BRAFi). In BRAF-mutated colorectal cancer, however, combinatorial therapy with an EGFR inhibitor (EGFRi) in addition to a BRAFi is necessary to achieve a relevant response (grey boxes). *BCR–ABL* translocations are considered a distinct hallmark feature of chronic myeloid leukaemia; strikingly, the recent report of a case of *BCR–ABL* translocation-positive glioblastoma and its successful treatment with imatinib shows that the *BCR–ABL* translocation can occur in tissues other than haematopoietic cells — albeit rarely — and can be effectively targeted[133] (red boxes). *BCR-ABL is found almost exclusively in leukaemia; however, a case report of it in glioblastoma has been published[133].

untargeted clones now benefitting from a competitive advantage and thus the tumour as a whole. This mixed response is an important conundrum in precision oncology, for which possible interventions can be designed. For example, co-targeting wild-type proteins from pathways presumed to be unaffected by oncogenic mutations could be an interesting experimental approach to raise pressure on a tumour in its totality, although the toxic effect on normal tissue elements might be limiting.

The effect of targeting specific mutations on the overall phenotypic appearance of a tumour remains to be fully elucidated. Seminal work in myeloproliferative neoplasms suggests that the order in which a given set of mutations are acquired strongly influences the phenotypic disease outcome[66] and therefore might be of importance during the targeted treatment of one or more genomic aberrations. Further, the concept of hierarchy implies that mutations with a higher allele frequency might be more important to a tumour than those with a lower allele frequency, although this ignores the possibility that interclonal competition can easily reverse this balance. Insights provided by sequential sequencing efforts such as by the TRACERx consortium in lung or renal cancer will be of the utmost importance in disentangling the impact of various clonal events and their resulting interdependency during therapeutic targeting[24,66–69].

Finally, as different clones may bear both overlapping and distinct molecular alterations[70], the concept of targeting convergent pathways may be called into question as alterations activated in different clones cannot converge. Single-cell molecular analysis may help uncover when alterations co-occur in the same cell or are derived from different cells. In the latter case, the treatment solution might involve directly targeting the altered gene product or products with combinations of drugs optimized for disrupting key malignant clones.

Riddle 5: How well should oncologists know their patients?

'Everything must be taken into account. If the fact will not fit the theory — let the theory go.'
Agatha Christie (The Mysterious Affair at Styles)[64]

Granular studies of host–tumour relationships with special attention to the host's genotype will be of critical importance for understanding the role of specific genomic variants in cancer. The question as to whether incorporating a patient's genomic background would benefit a therapeutic intervention remains poorly addressed, but it seems reasonable to hypothesize that genomic background, which influences immunity, toxicity and mutational function, should be important[71–76]. Oncologists have started to consider patient demographics (Fig. 5) more deliberately as it is becoming clear that cancer incidences and the genetic landscapes of tumours differ substantially across different genetic backgrounds and geographical locations, likely owing to different germline predispositions and exposure to different infectious and noxious agents. Some of the best-characterized examples of carcinogenesis being influenced by genomic background and geographical location are the differing distribution of driver mutations in head and neck cancers or cervical cancers caused by viruses[77,78] and oesophageal cancer across different countries[79]. The reasons for many demographic differences, such as the high incidence of tyrosine-kinase mutations in young female non-smokers of Asian origin[80], remain obscure. In part, progress in understanding how a patient's genetic background influences cancer progression has been hampered by the poor representation of non-white patients and patients from minority ethnic groups in sequencing efforts[81,82]. Considering the biological sex and age will be critical to optimizing precision oncology as therapy response rates and incidences differ across sexes[83–86], but the therapeutic implications of these factors have not been fully explored. Lastly, individual-specific lifestyles such as those associated with smoking or specific Western diets might fundamentally increase the risk of developing cancer, and the consequences of these lifestyles should be explored with regard to tumour genetic landscapes and sequencing-informed therapies.

One key extratumoural variable that has attracted attention during the past decade is the microbiota[87]. The microbiota, including bacteriophages, other viruses, fungi and bacteria, has been associated with a plethora of diseases[88]. Many correlative associations have been published with regard to cancer[87,89,90]. Although functional studies linking specific microbiota species with phenotypes remain rare, interesting clinically actionable associations have been made. For example, gut microorganisms have been reported to modify hormonal metabolism, thereby modulating the evolution and therapeutic response of prostate cancer[91]. Furthermore, the microbiota might play a role in determining the outcome of specific genomic abnormalities, such as the effect of *TP53* mutations, which can be tumour promoting or tumour

suppressing depending on the local flora[92,93]. The intestinal microbiota might also affect the outcome of checkpoint blockade and chimeric antigen receptor T cell immunotherapy[94,95], and there is now ample evidence that the abundance of specific bacterial taxa such as *Akkermansia* are associated with favourable immunotherapy outcomes[96]. Further, studies linking the efficacy of checkpoint therapy with the microbiota have been published for epithelial tumours and melanoma[97,98]. Today, the importance of the microbiota for the efficacy of immunotherapy has been increasingly accepted[99], and the therapeutic effect of restoring a beneficial microbiota has been suggested in seminal trials[100–102].

Riddle 6: What is the right time for immunotherapy?

'The impossible cannot have happened, therefore the impossible must be possible in spite of appearances.'
Agatha Christie (Murder on the Orient Express)[103]

One of the first clinical observations with regard to immunotherapy in cancer was made at the end of the nineteenth century by the American surgeon William Coley[104], who noted that injecting erysipelas cultures into patients with cancer would lead to a reduction in tumour burden. Although this strategy was quickly abandoned, the overarching principle of harnessing the immune system to fight cancer has become one of the brightest hopes in oncology. The emergence of human immunodeficiency virus (HIV) infections confirmed that the immune system impairs cancer development as patients with acquired immunodeficiency syndrome are predisposed to cancers such as Kaposi sarcoma, a tumour of endothelial cells induced by human gammaherpesvirus 8 that can shrink on the initiation of treatment of HIV infection and is sensitive to checkpoint blockade immunotherapy[105,106]. Interestingly, patients with other types of severe immunodeficiency are susceptible only to specific cancers; for example, patients with chronic mucocutaneous candidiasis are susceptible to oral cancers and squamous cell carcinoma[107], demonstrating the efficacy of the immune system in eliminating many nascent cancer clones even when weakened.

A key achievement in cancer immunotherapy has been the approval of the anti-PD1 antibody pembrolizumab for tumours with high tumour mutational burden (TMB) and/or microsatellite instability/mismatch repair genetic defects[108,109]. Although previously considered untreatable by conventional targeted therapies owing to their high

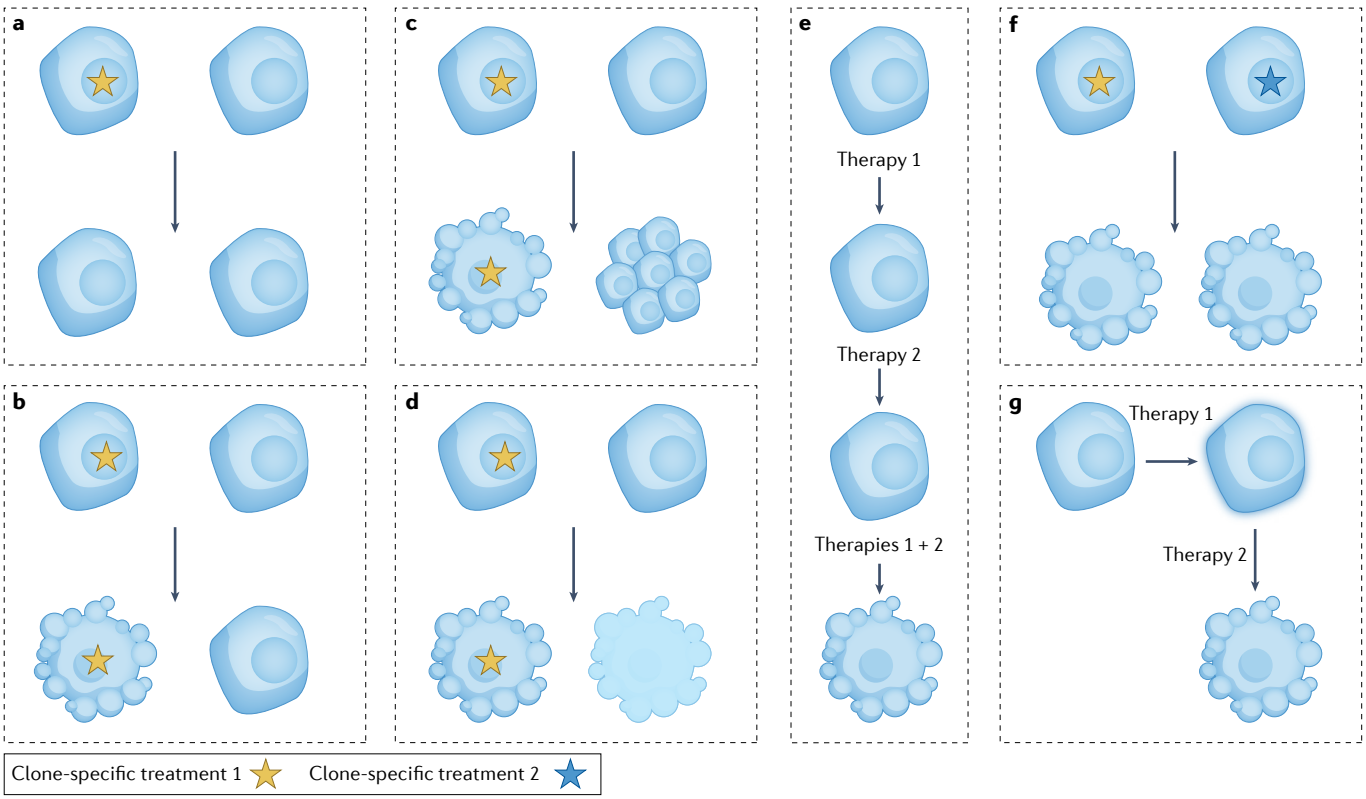

Fig. 4 | Which tumour clone should be targeted? Targeted treatment of clonal events can result in heterogeneous scenarios depending on the relevance of respective genomic lesions and the growth dynamics of different clones within a tumour. **a**, Targeting a genetic alteration of indeterminate relevance for tumorigenesis might have no effect whatsoever. **b**, Ideally, targeting one clone with molecularly targeted therapy should lead to its growth suppression and hence tumour regression. **c**, If interclonal competition maintains an equilibrium that is destabilized by a singular targeted therapy, outgrowth of the untargeted clone might ultimately lead to a reduction in clinical benefit, even though the intended target was suppressed. **d**, Therapies possessing a bystander effect, such as antibody–drug conjugates, efficiently target cells carrying the respective biomarker as well as cancer cells in close proximity. **e**, While a tumour might not respond to individual therapies, a combination of two drugs can result in efficient antitumoural effects owing to synthetic lethality. **f**, Targeting two clones individually may prevent accelerated growth owing to a competitive advantage as described for panel **c**, but could be limited by toxicity. **g**, Seemingly ineffective therapies might theoretically sensitize tumours to following treatments applied in a sequential treatment approach.

volume of genomic alterations, these tumours are the most likely to respond to immune checkpoint blockade. Some of these responses are remarkable, with durable complete remissions in patients with otherwise refractory disease. The underlying biology behind the responsiveness of high-TMB tumours might be that multiple mutations can exist only through the exploitation of a checkpoint that inactivates the immune system and that once this checkpoint is blocked with an inhibitor, the immune system is reawakened. Further, tumours with a higher mutational burden are likely to present more mutanome-derived neoantigens to T cells through human leukocyte antigen, thus increasing tumour immunogenicity[109,110]. However, we lack a precise understanding of the other factors that might play clinically relevant and/or actionable roles in enabling efficacious immunotherapy for cancers.

Although high TMB and PDL1 expression have gained importance in clinical decision-making, the use of immunotherapeutics should be accompanied by refined diagnostic criteria to ensure the right checkpoint is targeted[111,112]. Mutation-derived neoantigens must be immunogenic – a factor shaped by mutational signatures and immune responsiveness[113,114] – and the patient's major histocompatibility complex must be able to adequately present the neoantigens[115]. Further, the T cell receptor repertoire, which may be limited by factors such as neoantigen resemblance to self, must be able to recognize the presented neoantigens[116,117]. Clinically relevant approaches that try to exploit the occurrence of neoantigens in a personalized and unbiased fashion could improve the effectiveness of T cell-based immunotherapies and are currently at an experimental stage[118].

The efficacy of checkpoint blockade in cancers with high TMB contrasts with that of gene-targeted therapy, which appears to be most successful in cancers such as CML that are driven by a single gene alteration. However, immunotherapy can show high efficacy in conjunction with gene-targeted therapy[119,120]. It is unclear whether immunotherapy in these cases works additively or synergistically with the other therapies given or whether different subsets of patients are impacted differentially by the agents in these combinations.

Finally, first-line immunotherapy is being approved for an increasing number of cancer types, with the most experience obtained in non-small-cell lung cancer[121]. In a recent study examining the effect of using the anti-PD1 antibody dostarlimab as a neoadjuvant treatment in patients with rectal cancer, all 12 patients achieved complete remission, suggesting that moving immunotherapy to earlier in the course of the disease warrants additional investigation[122].

Conclusions

'Our weapon is our knowledge. But remember, it may be a knowledge we may not know that we possess.'
Agatha Christie (The A.B.C. Murders)[123]

The key aim for cancer treatment is the development of therapies that exclusively target cancer cells without causing harm to normal tissue. Two major approaches have begun to realize this goal: gene-targeted therapies and immunotherapy. These approaches are distinct in strategy and in the type of cancers most susceptible to them. Gene-targeted therapies impact a specific genetic alteration in the cancer and are most effective in early cancers before the occurrence of genomic evolution yields additional co-drivers. The poster child for successful gene-targeted therapy is CML, a previously lethal leukaemia that now has a life expectancy close to normal. The success of therapy for CML required three ingredients: the discovery of the underlying molecular genetic defect (the *BCR–ABL* fusion gene); development of a therapy that attenuates the enhanced kinase activity resulting

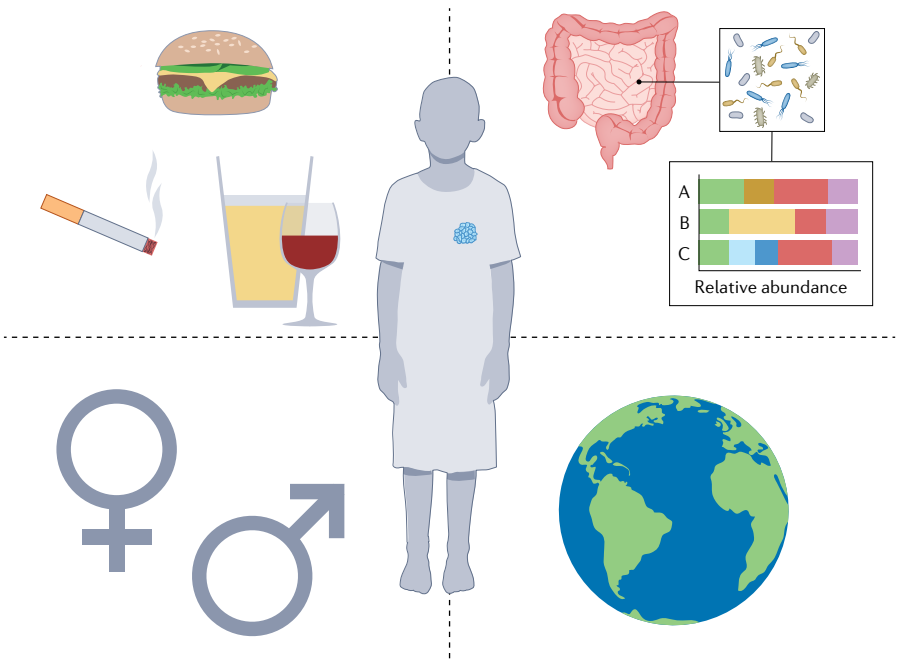

Fig. 5 | How well should oncologists know their patients? It has been shown that diet and lifestyle, the gut microbiota and exposure to bacteria and viruses, sex, and the geographical and ethnic origins of a patient affect cancer demographics, treatment response and the frequency of distinct molecular alterations. To treat a patient with personalized, optimized precision therapy, these factors must be taken into consideration.

Box 2

Financial and equity hurdles of new technologies

Ever since the US National Human Genome Research Institute estimated that the cost decline for sequencing a single human genome had beaten Moore's law, the common perception is that sequencing is affordable and, therefore, widely available. In mid-2021, the average cost per genome was approximately $500, or $0.005 per megabase[134,135]; however, these price estimations represent benchmark values, rarely applicable in a real-world setting. Moreover, the rate of decline in sequencing costs has reached a plateau phase in recent years. This poses a challenge in achieving high-coverage sequencing of individual patients and drives costs for liquid biopsy-based cell-free DNA analysis, a technology critical to monitoring the success of molecularly targeted therapy[137,138]. Additional new technologies beyond tissue genomics such as transcriptomics, single-cell analysis, epigenomics and functional assays all further add to costs and face technological hurdles such as complicated sample preparation to make them accessible and with rapid readouts.

The high costs of therapy and sequencing hamper the global adoption of successful therapeutic approaches in precision oncology. Evidence from developing countries suggests that the achievement of durable responses in chronic myeloid leukaemia can be attained only when clinical care is embedded in a modern and financially stable health-care system[139]. Indeed, recent clinical studies from East Africa showed that delays in diagnosis and difficulties in accessing required drugs hampered the treatment of chronic myeloid leukaemia in this setting[140,141]. Altogether, these aspects show how financial and technological hurdles still hamper the broad adoption of molecularly targeted therapy and raise the need to test ways in which the wider accessibility of next-generation sequencing and therapy could improve therapeutic outcomes.

from this defect (imatinib and second-/third-generation BCR–ABL kinase inhibitors); and the application of the therapy (imatinib) in newly diagnosed disease that has not yet undergone genomic evolution. Study of solid tumours harbouring specific gene anomalies has yielded the ability to drug targets such as *KRAS*, previously deemed undruggable[124], and has thus recapitulated the first two ingredients for a successful therapy as seen in CML. However, beneficial impact on outcomes might be limited because such targeted therapies are generally applied to metastatic disease — a setting when the malignancy has multiple co-drivers — rather than in newly diagnosed disease. Success of future precision therapies will require the right drug or drugs be given to the right patient at the right time.

NGS of solid cancers has made it clear that advanced malignancies have multiple alterations that differ from patient to patient, which could limit the effectiveness of gene-targeted monotherapies. Major advances in such cancers will require interrogating them with techniques such as transcriptomics[125], which delve into biology deeper than genomics alone and allow customized combinations of therapy that reflect the granularity of the tumour's aberrant signalling or exploit the increased expression of surface markers using cellular therapies or antibody–drug conjugates[125–128]. A new class of trials — *N*-of-1 trials — is emerging, where each patient receives a tailored drug cocktail combination; as millions of combinations are possible, the efficacy of the strategy rather than that of the specific combination is measured[129–131].

Importantly, immune checkpoint blockade may be best suited to complex tumours with high mutational burden due to mismatch repair gene defects — precisely the cancers least likely to respond to gene-targeted treatments[34]. This is because immunotherapy works by reawakening the immune system, which was inactivated by the tumour for it to survive; once the immune system has been reactivated, the more riddled the cancer is with genomic defects that yield neoantigens, the more likely the immune system is to recognize the tumour as distinct from normal tissue elements and to target it for eradication.

Puzzles emerge as the exploration of the cancer genome is expanded. For example, putative oncogenic drivers are found in a variety of non-malignant conditions, which begs the question as to how to determine whether a deleterious gene alteration is actually pathogenic[33,34]. It also introduces the interesting possibility of repurposing cancer therapies for non-malignant illnesses. Other key questions in the context of cancer also arise regarding the role of the tissue of origin in cancer, how it shapes the biological implications of genomic aberrations, how the host's genome and other features shape treatment outcomes and — with abundant evidence of clonal heterogeneity[124,129] — which clones are best targeted. The full interrogation and treatment of malignancies will require individualized functional and phenotypic characterization of the host and the cancer and will exploit advanced analytic tools to determine how to best target the tumour while minimizing damage to the patient's normal tissue. Of note, other hurdles must be overcome to optimize the management of many cancers, including fully tapping the multidisciplinary expertise of molecular tumour boards, addressing the cost of sequencing, and managing the toxicity and cost of combination therapies (as well as the need to bring two or more companies together for the drugs to be obtained), as elaborated in Box 2. Finally, while the recent success of *KRAS*^{G12C} inhibitors is encouraging[124,132], precision oncology suffers from our continued inability to target tumour suppressor inactivation. Inhibiting gain-of-function alterations has proved much easier than replacing loss-of-function alterations. Novel strategies are needed for cancers driven by validated tumour suppressors, as well as important drivers such as gene fusions, whose oncogenic signalling impact in many cases remains unclear.

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