Synthetic lethality as an engine for cancer drug target discovery

Alan Huang[1], *Levi A. Garraway*[2,4], *Alan Ashworth*[3] *and Barbara Weber*[1]*

Abstract | The first wave of genetically targeted therapies for cancer focused on drugging gene products that are recurrently mutated in specific cancer types. However, mutational analysis of tumours has largely been exhausted as a strategy for the identification of new cancer targets that are druggable with conventional approaches. Furthermore, some known genetic drivers of cancer have not been directly targeted yet owing to their molecular structure (undruggable oncogenes) or because they result in functional loss (tumour suppressor genes). Functional genomic screening based on the genetic concept of synthetic lethality provides an avenue to discover drug targets in all these areas. Although synthetic lethality is not a new idea, recent advances, including CRISPR-based gene editing, have made possible systematic screens for synthetic lethal drug targets in human cancers. Such approaches have broad potential to drive the discovery of the next wave of genetic cancer targets and ultimately the introduction of effective medicines that are still needed for most cancers.

Cancer is a disease of the genome, and almost all cancers have multiple genetic lesions that must be addressed to develop curative combination therapies[1]. A reductionist view of the hallmarks of cancer suggests that targeting oncogenic drivers, tumour suppressor gene loss and the underlying mechanisms by which cancer cells evade immune destruction are the minimum that will be required for cures[1].

Completion of the Human Genome Project in 2003 (REFS[2,3]) followed by advances in sequencing technology and the analysis of thousands of human tumours[4–7] enabled discovery of the first generation of genetically targeted cancer therapies, which, even as single agents, changed the lives of many people with cancer. Imatinib, which targets the *BCR–ABL* fusion tyrosine kinase, has extended the median survival of patients with chronic myelogenous leukaemia to more than 10 years[8–11]. Imatinib also inhibits KIT and is effective in treating *KIT*-mutant gastrointestinal stromal tumours, with response rates of up to 50% and a median progression-free survival of approximately 18 months[12–15]. The BRAF inhibitor vemurafenib[16], followed a few years later by dabrafenib[17] and encorafenib[18], transformed *BRAF*-mutant melanoma from an untreatable, rapidly progressive malignancy to a disease in which more than 50% of patients have meaningful clinical responses[19,20] and when combined with a MEK inhibitor, have a median progression-free survival of approximately 12 months with limited toxicity[21–23]. There are multiple other examples of clinical successes, including drugs that target amplified *ERBB2* (encoding HER2) in breast cancer[24,25], *EGFR* mutations and *ALK* translocations in non-small-cell lung cancer (NSCLC)[26–35] and numerous others[36]. However, our ability to make further progress with genetically targeted cancer therapy has been limited by two main issues. First, although partial responses to targeted therapies in selected patient populations are common, converting those partial responses to durable complete responses, which is needed for cures, will require combination regimens that have been challenging to define. Additionally, although DNA sequencing technology has enabled the identification of most, if not all, oncogenes that arise as a consequence of genetic alterations[37–39], they represent a relatively small percentage of genes that are relevant in cancer, not all of which are druggable with conventional approaches. Proteolysis-targeting chimaeras (PROTACS) and other protein degradation approaches are likely to change the definition of 'druggable' in the coming years[40,41], but independent of advances that may redefine druggability, identification of the next wave of cancer drug targets requires more than deep sequencing of multiple tumours. CRISPR-enabled functional genomic screening platforms are powerful tools for this application.

As enthusiasm for targeted cancer therapy waned somewhat in the face of its limitations, progress harnessing the immune system to treat cancer allowed another wave of clinically meaningful responses, and, in some cases, cures. Many patients with melanoma, regardless of *BRAF* mutation status[42–44], renal cell carcinoma[45], NSCLC[46–49] and a number of other cancers[50] have clinically meaningful responses to checkpoint inhibitors

[1]*Tango Therapeutics, Cambridge, MA, USA.*
[2]*Eli Lilly and Company, Indianapolis, IN, USA.*
[3]*UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, USA.*
[4]*Present address: Roche/Genentech, South San Francisco, CA, USA.*
**e-mail: bweber@tangotx.com*

https://doi.org/10.1038/s41573-019-0046-z

such as anti-programmed cell death 1 (PD1) or anti-PD1 ligand 1 (PD-L1), as well as in some cases antibodies against cytotoxic T lymphocyte antigen 4 (CTLA4), alone or in combination with another checkpoint inhibitor[51,52]. These important successes further dampened enthusiasm for genetically targeted therapies and spurred a wave of clinical investigation in a broad range of tumour types with many putative immune cell targets. This next wave of immunotherapy agents has yet to prove as effective as anti-PD1 and anti-PD-L1 agents[53], and it remains to be determined whether immunotherapies will do what the initial targeted therapies failed to do: to produce durable complete responses and cures in large numbers of patients with cancer.

Therefore, although advances in both targeted therapy and immunotherapy have improved cancer treatment for many people in remarkable ways, barriers to targeting tumour suppressor gene loss, identifying context-dependent driver genes that are not marked by genetic alterations (which result in non-oncogene addiction[54,55], called here 'unmarked oncogenes'), designing the next generation of novel combinations and exploring the largely unknown genetics of tumour-intrinsic immune evasion have stalled progress. However, the genetic principle of synthetic lethality coupled with the power of CRISPR-based functional genomic screening technology offers a path forward.

Synthetic lethality, initially described in *Drosophila* as recessive lethality[56], is classically defined as the setting in which inactivation of either of two genes individually has little effect on cell viability but loss of function of both genes simultaneously leads to cell death. In cancer, the concept of synthetic lethality has been extended to pairs of genes, in which inactivation of one by deletion or mutation and pharmacological inhibition of the other leads to death of cancer cells whereas normal cells (which lack the fixed genetic alteration) are spared the effect of the drug. In the most straightforward application, this means identifying targeted therapies that kill cancer cells that lack a specific tumour suppressor gene but spare normal cells. In addition to this (conceptually) simple application, the tools needed to discover synthetic lethal interactions in human cancer cells can be applied in multiple ways to identify a number of other types of cancer drug targets.

In this Review, we discuss how the genetic concept of synthetic lethality paired with CRISPR-based functional genomic screening can be applied to identify the next generation of effective cancer drugs and combinations. Although much has been written about synthetic lethality in cancer since Hartwell, Friend and colleagues raised the idea in 1997 (REFS[57–59]), application of the concept to cancer drug discovery has been largely aspirational. This is largely because of the limitations of using yeast and *Drosophila* as model organisms for human disease and the limitations of previous generations of genetic tools, such as RNA interference (RNAi), for mammalian studies. The widespread availability of CRISPR-based tools and the increasingly varied ways they can be used has created an inflexion point that has the potential to dramatically alter cancer target discovery and create a wave of new therapeutics in the next decade. Here we analyse the evidence that many cancer targets remain to be discovered using a CRISPR-based functional genomic screening and how this approach can lead to the discovery of additional targets based on loss of tumour suppressor genes. We also highlight the importance of genetic context in designing target discovery strategies, and analyse the technical considerations for scalable synthetic lethal target discovery, including the relative benefits of various CRISPR-based tools and libraries, discussing inhibition of protein arginine *N*-methyltransferase 5 (PRMT5) in cancers with deletion of *S*-methyl-5′-thioadenosine phosphorylase (MTAP) as an example of targeting synthetic lethality beyond poly(ADP-ribose) polymerase (PARP) inhibitors in *BRCA1*-mutant and *BRCA2*-mutant contexts. Finally, we discuss the applications of CRISPR-based screening with targeted drugs for novel combination discovery and in vivo screening for non-cell-autonomous mechanisms.

Synthetic lethality

The genetic concept of synthetic lethality was first described in *Drosophila* in 1922 (REFS[56,60–62]). Fruit flies with individual abnormal eye phenotypes (phenotypes that were attributed to either *Bar* or *glass* mutations) could survive and reproduce but viable offspring with a combination phenotype were never observed[56]. We now know that *Bar* and *glass* encode transcription factors expressed in the eye, an extension of the central nervous system, and are involved in embryonal development. We can therefore postulate that loss of both genes simultaneously results in neural development defects not compatible with life, although the specific lethality mechanism of this synthetic lethal pair has not been studied directly. The concept was subsequently also shown to be relevant in yeast[63], and eventually was proposed as a basis for drug discovery for human disease by Hartwell, Friend and colleagues 20 years ago[57]. Hartwell, and subsequently Kaelin, proposed that novel targets for cancer therapeutics could be discovered by exploiting the concept of synthetic lethality, in which one member of a synthetic lethal pair is a gene product with a cancer-specific mutation and the second gene product is the drug target[57,58].

The systematic identification of synthetic lethal pairs relevant to human disease was initially limited to loss-of-function screens in model organisms. For example, genetic interaction maps in yeast were readily generated by genetic screening and crossing of knockout strains[57,63–65], but the utility of these findings for cancer drug discovery relies on the existence of relevant homologues in human cells. The advent of RNAi technology allowed the broader application of these concepts to human cell line systems, and a number of subsequent screening efforts in cancer cells were undertaken[66]. Proof-of-concept classical synthetic lethal screening using small interfering RNA (siRNA) to identify dependencies conferred by loss of the tumour suppressor gene *VHL* (encoding von Hippel–Lindau disease tumour suppressor) in clear cell renal carcinoma was described in 2008 (REF.[67]). Further application of systematic RNAi screening led to the discovery of additional synthetic lethal pairs, including members of the SWI/SNF chromatin remodelling complexes such as *SMARCA2* and

Genetic context
Histology and genetic architecture that define a specific set of cancer patients (for example, patients with *BRCA1*-mutant ovarian cancer).

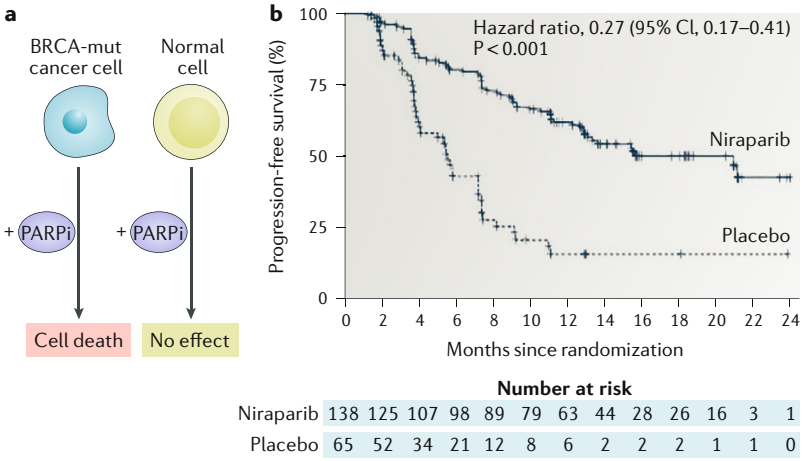

Fig. 1 | **Synthetic lethality: a genetic concept reduced to clinical practice.** Schematic (part **a**) and phase III clinical data[197] (part **b**) showing a clinical example of the utility of synthetic lethality for drug development. Poly(ADP-ribose) polymerase inhibition (PARPi), which has minimal effect in normal cells with wild-type BRCA gene function, causes tumour cell-specific death in BRCA gene-mutated tumour cells, significantly extending the progression-free survival of patients with germline mutations in BRCA genes. BRCA-mut, BRCA gene mutant. Part **b** from REF.[187], *N. Engl. J. Med.*, Mirza et al., Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer, **375**, 2154–2164. Copyright © 2016, Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

SMARCA4 (REFS[68–70]) and *ARID1A* and *ARID1B* (REF.[71]), in addition to the PRMT5–MTAP interaction[72–74] discussed in detail later in this Review.

The recent success of PARP inhibitors in BRCA-mutant ovarian cancers is the first clinical example of using synthetic lethality to target tumour suppressor gene loss[75–79] (FIG. 1). The basis for this finding is that both PARP and BRCA1 and BRCA2 are components of efficient DNA repair. This interaction makes tumour cells with mutations in *BRCA1* or *BRCA2* sensitive to PARP inhibition, driving efficacy. Normal cells, which have at least one copy of *BRCA1* or *BRCA2*, are largely spared, which limits toxicity. It now has been well described that all PARP inhibitors that have reached clinical stages have both catalytic inhibitory and DNA trapping activity[80,81], which has led to some controversy regarding the mechanism of lethality. Whereas the DNA trapping activity of PARP inhibitors clearly enhances the effect of genetic knockdown of PARP, the activity of PARP inhibitors in tumours with *BRCA1* or *BRCA2* mutations is tightly linked to loss of function of the gene products. Furthermore, it has been shown that PARP inhibitors with markedly different DNA trapping potencies have comparable activity as measured by growth inhibition at maximum tolerated doses in xenograft models of *BRCA1*-mutant triple-negative breast cancer[82]. As PARP inhibitors are used at doses that maximize both PARP catalytic inhibition and DNA trapping activity, the relative importance of those functions in clinical response remains unknown. Therefore, although the exact mechanism underlying PARP–BRCA1 and PARP–BRCA2 synthetic lethality remains unclear, DNA damage repair as the basis for the interaction remains undisputed. The FDA has approved four PARP inhibitors for use in patients with BRCA-mutant cancers (olaparib, rucaparib, niraparib and talazoparib)[76]. Of note, PARP inhibitors seem more effective in *BRCA1*-mutant and *BRCA2*-mutant ovarian cancers than in breast cancers with these mutations, which raises the consideration of additional 'genetic context', a critical factor in designing functional genomic target discovery screens, as discussed later.

Many cancer targets to be discovered

Large-scale gene-knockout studies across many genetic contexts are now being used to map synthetic lethal interactions in human cancer cells — first using short hairpin RNA (shRNA)-based approaches and more recently using CRISPR technology, which has eliminated many of the technical hurdles of RNAi-based functional genomic screens[83–85] (BOX 1). Project DRIVE (conducted at Novartis) and Project Achilles (conducted at the Broad Institute) use the Cancer Cell Line Encyclopedia — a large panel of human cancer cell lines that represent multiple cancer types[86] — to create a catalogue of essential genes and synthetic lethal interactions[87–91]. The Sanger Institute has taken a similar approach in 324 human cancer cell lines from 30 cancer types (Project Score)[92]. The number of novel druggable targets that have been nominated and fully validated by Project Achilles and Project DRIVE has thus far been limited, with PRMT5 (REFS[72–74]) and Werner syndrome ATP-dependent helicase (WRN)[92–95] being the best examples. However, Project Score focused specifically on identification of additional novel drug targets, and has provided convincing evidence that many undiscovered targets indeed exist, that most are context dependent, and that they can be discovered with a functional genomics approach[92].

The Sanger Institute effort, recently described by Garrett and colleagues, includes 941 CRISPR–Cas9 screens in 339 cell lines from the Cell Model Passport collection using a genome-scale library targeting ~18,000 genes[92]. The final analysis included 324 of those cell lines. Genes required for cell fitness (called 'core fitness genes' or 'essential genes') across the majority of cell lines were deemed unlikely to be good drug targets due to a narrow therapeutic index. In total, 7,470 targeted genes (41%) altered cell viability in at least one cell line (BOX 2). Good drug targets, likely to be represented by fitness genes restricted to specific molecular contexts or histologies, were defined as a subset of those genes with fitness effects in 12 or fewer of the 13 cancer types included in the screen. With a defined median of 1,459 fitness genes per cell line, a median of 553 genes were considered pan-cancer fitness genes and 866 were nominated as cancer type-specific genes. To further refine priority drug targets, genes were scored for various measures of the effect of gene ablation, including extent of the effect, levels of expression of the target gene and mutational status (70% of the priority score), as well as evidence of a genetic biomarker associated with the target dependency and the somatic alteration frequency of the target gene in human cancers (the remaining 30% of the priority score). With this paradigm, 617 priority cancer type-specific targets and 92 priority pan-cancer targets were defined. Most of the cancer type-specific

priority targets were identified in two or fewer cancer types ($n = 457$; 74%), emphasizing the importance of context. Finally, a tractability filter for the development of small molecules and therapeutic antibodies was applied. Tractability group 1, which included 40 genes (6%), included all targets of drugs approved or already in development. A large number of priority targets, 277 genes (44%), fell into tractability group 2, defined as targets with no known development efforts but with evidence supporting target tractability. Finally, the largest number of putative targets, 311 (48%), were deemed untractable by conventional means. Although these data do not suggest that all genes with a fitness effect are good drug targets, or that all undiscovered targets are represented in this analysis, they provide strong evidence for the presence of a large target space remaining to be explored and many paths to discover effective new treatments for patients with cancer.

Given the evidence from the Sanger analysis that a large number of druggable cancer targets remain to be discovered, why have so few emerged from other large-scale experiments thus far? The answer likely lies in both the primary aim of Project Achilles and similar projects[87,92] and their design. Although novel drug target discovery is a potential benefit of Project Achilles, the overarching goal is to increase our understanding of cancer biology. The data obtained are extremely valuable for many applications, but efficient and comprehensive drug target and combination discovery requires some refinements tailored to specific applications.

First, although Project Achilles and Project DRIVE have included more than 300 cancer cell lines so far, there are many cancer subtypes insufficiently represented for context-specific analyses and some histologies are not represented at all. For example, in the collection there are no cell lines for human papillomavirus-positive head and neck cancer, which represents more than half of all newly diagnosed head and neck cancer cases in the United States[96] and, whereas there are more than 90 NSCLC cell lines, there is only one cell line each representing *ALK* translocation or *MET* amplification, and there are no cell lines with *MET* exon 14 skipping mutations[87,90]. *ALK* translocations are present in less than 5% of lung cancers overall[97], but they are disproportionally represented (40%) in younger non-smokers (<40 years) with lung adenocarcinoma[98]. As in the case of *ALK* mutations, genetic alterations in *MET* are similarly uncommon in lung cancer overall but represent a very important therapeutic target in a similar proportion of patients[97]. Second, the intentional heterogeneity of the Cancer Cell Line Encyclopedia collection introduces a mathematical challenge to identifying even strong signals when they are present in a small subset of the collection. This is the same problem encountered in clinical trials of targeted agents conducted without patient selection: if the number of patients in the trial with the genetic context necessary for response is small, even a large signal in individual patients will be diluted by non-responders, and the trial will fail. An analogous problem occurs when one is analysing large heterogeneous panels of cell lines: a strong signal from a small number of cells will be lost. This problem was addressed analytically in Project Score, revealing the strength of the effect: of the 628 priority targets identified in the analysis, 56% had a fitness effect in only one cancer type and an additional 18% had a fitness effect in only two cancer types. Third, the readout for Project DRIVE and Project Achilles screens is guide RNA (gRNA) ratios, which reflect growth rate changes and/or cell death from in vitro monocultures, eliminating the ability to discover non-cell-autonomous mechanisms such as immune evasion. Finally, the screens are conducted in vitro with CRISPR or shRNA libraries as the sole perturbation, and thus the data cannot be used to identify novel combination therapies because only one gene is perturbed in each cell and no drugs are used in combination with the genetic perturbation. These last two issues cannot be addressed analytically in any of the large datasets now available and require experimental designs specific to those types of targets.

Following the successful application of loss-of-function screening across large cell line panels and the discovery of CRISPR, a number of biotechnology companies have been founded in recent years to identify and prosecute novel synthetic lethal drug targets, and it is likely that a number of pharmaceutical companies have started to use this approach for target discovery when relevant model systems are available. The first wave of targeted therapies from these efforts is emerging, and it seems likely that the initial clinical trials from these efforts will start to enrol patients within the next few years.

The importance of genetic context

As noted already, considering genetic context is important to avoid signal dilution due to heterogeneity in both clinical trial design and experimental design. The two are tightly linked: the genetic context selected for target discovery should form the basis for a patient selection strategy in clinical development. In addition, many synthetic lethal pairs are likely to be context dependent, with alterations in other genes in specific contexts altering

Box 1 | **Big data approaches to synthetic lethal drug target discovery**

Project Achilles is a functional genomic screening initiative from the Broad Institute with the goal of creating a genome-wide catalogue of tumour vulnerabilities associated with genetic and epigenetic alterations. Short hairpin RNA (shRNA) screening and now CRISPR-based screening have been applied to cancer cell lines at the genome scale to interrogate gene essentiality, providing the foundation for a cancer dependency map[88–91]. Project DRIVE (deep RNA interference (RNAi) interrogation of viability effects in cancer) is a large-scale RNAi screen of almost 400 cancer cell lines led by Novartis to define cancer dependency genes. Project DRIVE was designed to overcome the inability of RNAi screens to distinguish between on-target and off-target effects when using low numbers of shRNAs per gene and to increase the statistical power to describe molecular correlates of knockdown effects. In a large-scale robotics approach, a lentiviral library targeting 7,837 human genes was produced with a median of 20 shRNAs per gene (compared with the standard use of three to five shRNAs per gene) and was used to screen 398 cancer cell lines in a pooled format[87].

Project Score is an initiative from the Sanger Institute to profile the genetic dependencies of 324 cancer cell lines across 19 tissues and 30 cancer types using a whole-genome CRISPR library targeting approximately 18,000 genes[92]. These data were specifically analysed to determine the number of novel, tractable drug targets that remain to be discovered, convincingly demonstrating that the number is likely in the hundreds. This dataset can be used to identify potential drug targets that are active in a predictable subset of cancer cell lines, as exemplified by discussion in the publication of Werner syndrome ATP-dependent helicase (WRN) as a target in microsatellite instable tumours.

Box 2 | **CRISPR screen in a large cancer cell line panel suggests many more drug targets to be discovered**

A panel of 324 cell lines from the Sanger Project Score was screened using a genome-scale CRISPR library and then analysed to nominate those genes important for cellular fitness. Of the genes tested, 41% impacted growth of at least one cell line (7,470 fitness genes represented in the figure), with most of these genes impacting a minority of the cell lines (falling below the 50% of cell lines mark), suggesting context specificity of the dependency[92]. Strong drug targets would be those that selectively kill a subset of cell lines with a predictable genetic feature and harbour a conventionally druggable protein domain.

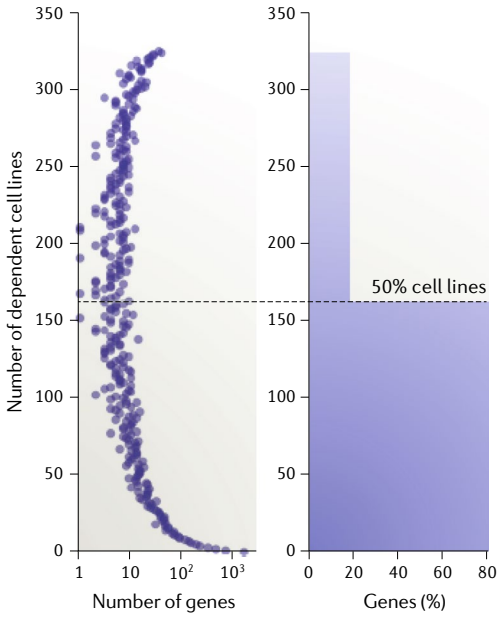

Figure reproduced with permission from REF.[92], Springer Nature Limited.

the functional interaction of the synthetic lethal pair. We define 'genetic context' as the driver mutation, tissue of origin (histology) and other functional genetic lesions that make up subgroups of cancers. The importance of genetic context is exemplified by the discovery of the selective dependency of ovarian clear cell carcinoma and melanoma cell lines on the oxygen sensor EGLN1 (REF.[99]). EGLN1 is a member of the EgIN family of prolyl hydroxylases that regulates levels of hypoxia-inducible factor 1α (HIF1α) via hydroxylation and subsequent degradation by VHL[100]. Hahn and colleagues linked a cancer dependency to HIF1α upregulation, consistent with the role of EGLN1 in regulating cellular HIF1α levels. They postulated that pharmacologic inhibition of EGLN1, which stabilizes HIF1α, reduces cellular fitness, leading reduced proliferation and cell death[99]. In a more focused analysis of just the ovarian carcinoma cell lines that were included in Project Achilles, Briggs et al. identified *ARID1A* as an additional dependency[101]. In this analysis, it is not possible to separate the effects of HIF1α upregulation and *ARID1A* mutation as all cell lines with ENGL1 dependency are characterized by HIF1α upregulation, and most of those have *ARID1A* mutations. *ARID1A* is mutationally inactivated in ~60% of ovarian clear cell carcinomas[102,103] and is frequently mutated in other histologies but, of note, analysis of the Project Achilles dataset in its entirety does not identify any druggable ARID1A synthetic lethal interactions (ARID1B, a paralogue of ARID1A, is a strong synthetic lethal partner with ARID1A[71] but is not considered conventionally druggable owing to lack of enzymatic activity or previously targeted domains). Briggs et al. showed that pharmacologic inhibition of EGLN1 selectively kills *ARID1A*-mutant ovarian cancer cells; thus, it is plausible that the combination of these two abnormalities is responsible for the dependency[101]. The data from both reports[99,101] suggest that EGLN inhibitors — currently used clinically to treat anaemia — may be effective in treating women with ovarian clear cell carcinoma. The therapeutic effect may be most effective in an *ARID1A*-mutant subset, but that hypothesis has not been tested in clinical trials.

Another recent example of a synthetic lethal drug target discoverable only through analysis of cell lines with a specific genetic context is DNA polymerase θ (POLQ) in *BRCA1*-mutant and *BRCA2*-mutant cancers. POLQ is a low-fidelity DNA polymerase that participates in alternative non-homologous end joining[104–107], a critical pathway for the repair of DNA double-strand breaks in tumours with defective homologous recombination[108,109]. POLQ knockdown reduces cellular survival both on its own and in combination with PARP inhibitors in a *BRCA1*-mutant and *BRCA2*-mutant context but not a wild-type context[108,109]. This target was not identified by the original Achilles and Project DRIVE analyses[87,89], but is mentioned in the Sanger analysis[92]. This is probably due to the fact that there was an under-representation of *BRCA1*-mutant and *BRCA2*-mutant cell lines, but then this target became easily discoverable in a small panel of curated *BRCA1*-mutant cancer cell lines paired with wild-type *BRCA1* isogenic derivatives[110].

KRAS mutations provide another example of the complexity of context: cell lines and tumour models with G12C or G12A mutations in *KRAS* are very sensitive to tyrosine-protein phosphatase non-receptor type 11 (PTPN11) inhibitors, whereas those with mutations at G13 and Q61 are not[111,112]. This occurs because oncogenic G12 variants, but not G13 and Q61 variants, are dependent on PTPN11-mediated GTP loading to promote downstream signalling[111]. Given that G12C and G12A mutations are enriched in NSCLC but are rare in colorectal and pancreatic cancer, there may be differences in sensitivity to PTPN11 inhibition on the basis of histology. Moreover, DRIVE data analysis suggests that cells with *KRAS* mutations that co-occur with *SMARCA4* or *KEAP1* loss of function are less dependent on KRAS than those with wild-type SMARCA4 or KEAP1 function[87]. These data again highlight the importance of considering additional genetic context in target discovery, and further suggest that in some cases mutation subtyping may be needed for patient selection.

Large-scale target discovery approaches

As noted already, functional genomic screens, specifically high-throughput loss-of-function screens that identify the pairwise effects of synthetic lethal gene pairs, have been envisioned as a path to identifying novel targets and combinations for many years[58,59]; however, pre-CRISPR technology was not sufficiently robust for these applications. Before the discovery of CRISPR[113–116], RNAi was the standard tool for loss-of-function genetic screens in mammalian cells[117,118]. Although initially very promising, it rapidly became clear that RNAi lacks the specificity for high-throughput applications. This problem is inherent to the technology, as stretches of shRNA and siRNA sequences (called 'seed sequences') can bind to and downregulate mRNAs unrelated to the

Isogenic cell line pairs
Cultured cell lines genetically engineered to have only a single genetic difference between them.

gene of interest, resulting in multiple, largely unavoidable, false positive hits in genetic screens[119–121]. These off-target effects can now be predicted and computationally filtered to improve interpretation of existing shRNA screening data[89]; however, unless 15–20 shRNAs per gene are used for screening, identifying true hits from shRNA screens remains challenging and largely impractical without large-format robotics[87]. By contrast, CRISPR is a highly specific, efficient and scalable genome-editing technology markedly outperforming RNAi-based reagents[83–85,122] and can be applied in high-throughput screens to discover novel drug targets[123,124] using multiple related approaches.

The initial discovery of CRISPR in microorganisms was based on the native bacterial enzyme Cas9 which excises segments of foreign DNA from the host genome[125–127]. The utility of this powerful system in human cells was quickly realized with the development of gene-editing tools that pair stable expression of bacterial Cas9 with sequence-specific gRNAs that guide the enzyme to excise precise DNA fragments from the human genome and at genome scale if desired[114–116], now sometimes called 'CRISPR cutting'. These remarkable advances opened the door to a variety of modifications, as well as the discovery of additional editing enzymes such as the endonuclease Cpf1 (REFS[128,129]). Many of these emerging genomic engineering approaches have a role in cancer target discovery, as described in more detail in BOX 3. As additional genetic tools and modifications emerge, target discovery approaches will continue to be enhanced.

Strategies for CRISPR library selection. When one is considering functional genomic target discovery screens using CRISPR-based tools, the primary determinants of size, cost and time are the number of cell lines to be screened and the library size. The determination of the number of cell lines largely depends on the purpose of the screen: useful information can be obtained with just one cell line or one isogenic pair, particularly when CRISPR screening is combined with a pharmacologic inhibitor, or hundreds of cell lines, as with Project DRIVE, Project Achilles and Project Score[87,90,92,130]. Isogenic cell line pairs have the advantage

Box 3 | **CRISPR technologies that enable synthetic lethal drug target discovery**

CRISPR
CRISPR technology uses a guide RNA with a target sequence of 20 base pairs in length to direct Cas9 to sequence-specific regions of the genome, resulting in the DNA cuts that lead to gene product loss of function[113–115]. In human cells, such an approach can be leveraged to systematically study the functional effect of loss of each gene in the genome[123,124]. In addition to Cas9, Cpf1 is a class 2 CRISPR system that relies on a single RNA-guided nuclease effector[128,129].

CRISPR interference
CRISPR interference (CRISPRi) uses catalytically inactive Cas9 ('dead Cas9', dCas9) fused to a Krüppel-associated box (KRAB) protein domain that interferes with transcription, suppressing gene expression rather than inducing double-strand DNA breaks[172–174]. CRISPRi has the advantage of more closely mimicking the effect of a pharmacologic inhibitor, which incompletely suppresses activity. As a result, CRISPRi may allow differentiation between the enzymatic effect of a gene product versus a potential scaffolding effect, the latter not being amenable to pharmacologic inhibition. However, the number of poorly characterized transcription start sites in the genome limits this application and has the potential to introduce false negative results in a large genomic screen.

Combinatorial CRISPR
CRISPR systems that allow interrogation of more than one gene per cell will be particularly valuable in defining novel drug combination regimens. The Cas9 and Cpf1 systems may both be used to this end. The Cpf1 system allows one-step direct cloning of concatenated gDNA and seems to have a substantially lower rate of recombination in excising the guide RNAs than Cas9 owing to the vector design[175–180]. Combinatorial CRISPR also can be applied for high-throughput isogenic testing, with the first position of the vector targeting a tumour suppressor gene and the second position targeting the druggable genome. This approach has the potential to dramatically scale the identification of synthetic lethal targets using well-controlled isogenic systems.

Base editing
Base editing also uses dCas9; however, the transcriptional repressor is replaced by a DNA deaminase, which results in base pair modifications that are precisely targeted[181–184]. This technology is particularly useful for rapid generation of isogenic pairs of cell lines, for example, changing the endogenous *KRAS* allele from mutant to wild type, or vice versa. Such a system is well controlled in comparison with traditional methods of overexpressing an exogenous expression construct and could therefore be very powerful for synthetic lethal-based target discovery.

RNA targeting
C2c2, Cas13b and Cas13d are the latest CRISPR systems reported[185–191]. These enzymes recognize and cut mRNA, making possible both reversible and scalable target gene modulation. Such an approach mimics small-molecule or antibody drug modes of action better than DNA editing, as pharmacologic inhibitors rarely fully inhibit the target gene product, suggesting these will be powerful tools for drug target discovery.

Single-cell sequencing of pooled CRISPR screens
The complexity of the readout of pooled CRISPR-based screens can be greatly expanded by the application of single-cell sequencing to determine transcriptional activity and genetic perturbation in each individual cell[192–196]. Such an approach allows understanding of heterogeneity in a cell population as well as the elucidation of more complex mechanisms, beyond the viability readout that has historically been applied. This additional information is likely to be very useful for the prioritization of potential drug targets.

of being well controlled as the only difference between them is the removal of a single gene; however, this is a highly engineered system that may not recapitulate gene function observed in tumours from patients. With a cell panel approach for novel target discovery in the context of a tumour suppressor loss, five to ten well-matched lines will often usually provide sufficient confidence to support subsequent validation. Dependency surveys across the genetic spectrum of human cancer, such as Project Achilles, Project DRIVE and Project Score, will, however, require very large numbers of cell lines and are possible only with significant resources.

Once the number of cell lines to be screened has been determined, gRNA library size is the primary determinant of the resources required. The potential advantage of reducing library size without limiting actionable findings can be appreciated when one considers the method used for most large-scale CRISPR-based screens. These screens are usually conducted in a pooled format to allow maximum throughput with multiple internal controls. A library of gRNA constructs, usually packaged in lentivirus, is used to infect target cell lines and the effect of each gene knockout on cell growth can be individually assessed using next-generation sequencing. After an appropriate interval (usually 1–3 weeks) the abundance of each gRNA is measured: guide sequences associated with loss of cell viability will be depleted in the postinfection pool compared with their abundance in the preinfection pool. As library size is not a technical limitation for pooled screening, exome-wide interrogation is feasible for a limited number of cell lines without access to large-format robotics. By contrast, for plate-based screens, a tenfold increase in gRNA library size, such as that between a 500-gene kinome and a 5000-gene druggable genome, requires sophisticated automation equipment for a robust scale-up. However, even for pooled screening, library size does define the total number of cells required for each screen and can result in rate-limiting amounts of tissue culture when multiple cell line panels are screened with a large library for pooled screens. Larger cell line panels are important to adequately power a comparison between wild-type and mutant cell lines and account for secondary genetic changes present in all cancer cells. Therefore, limiting the size of the gRNA library size does not limit target discovery if the number of genes interrogated in a single screen is also limited at the same time. Moreover, in case of fixed resourcing, limiting the size of the gRNA library allows analysis of a larger number of cell lines with the same amount of tissue culture resource, which in turn adds statistical power to target discovery efforts.

When the experimental goal is drug target discovery, efficiency and productivity are important considerations, and several options may be considered. Kinome and phosphatome libraries have been used effectively for this purpose, but although they are manageable in size (518 and 298 genes respectively) many novel targets will fall outside these target classes. Project Score effectively used a genome-scale library to identify almost 300 putative novel druggable targets, but used a postscreen filter to eliminate genes that are considered undruggable by conventional methods[92]. Although this approach is analytically straightforward, a more efficient approach could be to use a 'druggable genome' library, effectively inserting that filter before the screen, and therefore markedly reducing the resources and time needed to generate and analyse the data. On the basis of the historical success of target classes, sequence-based and structure-based prediction, catalytic activity, and capacity of binding of an endogenous ligand, some studies have estimated that no more than a quarter of the human genome is druggable by conventional means (~5,000 genes)[131–133]. These analyses suggest that using a druggable genome CRISPR library will eliminate more than 70% of screen hits that are not conventionally druggable when a whole-genome library is used, allowing time and resources to remain focused on other critical activities relevant to target discovery, including screening in a broader panel of context-specific cell lines and subsequent target validation strategies. Some studies have successfully adopted such an approach by using a set of druggable genes defined as desired by the user[132,134,135]. One important consideration when one is constructing a druggable genome library is the value of adding a set of additional, conventionally undruggable genes relevant to cancer biology, such as the MAPK signalling pathway and all known recurrently mutated genes in cancer. The undruggable genes add great value by placing novel targets identified by screening into known biological pathways. They also provide both validation for hits and biological rationale when one is selecting potential drug targets for validation. Regardless of the definition, druggability is in the eyes of the beholder, new drug discovery paradigms such as induced proteolysis-targeting chimaeras are emerging[40,41] and the scope of any druggable genome library will need to be revisited as technology develops.

Targeting tumour suppressor gene loss

Tumour suppressor gene loss is a central mechanism by which normal cells undergo malignant transformation, often by causing or allowing genome instability. Many tumour suppressor genes have been well characterized, such as *TP53*, *RB1* and *BRCA1*, but tumour suppressor gene loss is by definition undruggable, as the function and often the genes themselves are lost. Identification of druggable synthetic lethal partners is currently the only way of targeting the functional loss of tumour suppressor genes in cancer.

As noted earlier, the discovery of PARP inhibition and *BRCA1* or *BRCA2* mutation as a synthetic lethal interaction by both Ashworth and colleagues and Helleday and colleagues in 2005 (REFS[75,79]) is the first to be translated into clinical benefit for patients. However, this discovery was not made by genomic screening, rather it was hypothesis-driven[76,79]. It was also blessed by a bit of good luck: a small-molecule PARP inhibitor had already been developed with plans for use as a cytotoxic agent, the role of BRCA1 and BRCA2 in DNA damage response was known, and the synthetic lethal effect of PARP inhibition with BRCA loss is very strong[74]. However, the initial enthusiasm that additional hypothesis-driven efforts would identify druggable synthetic lethal partners for other DNA damage-related genes has not yet been

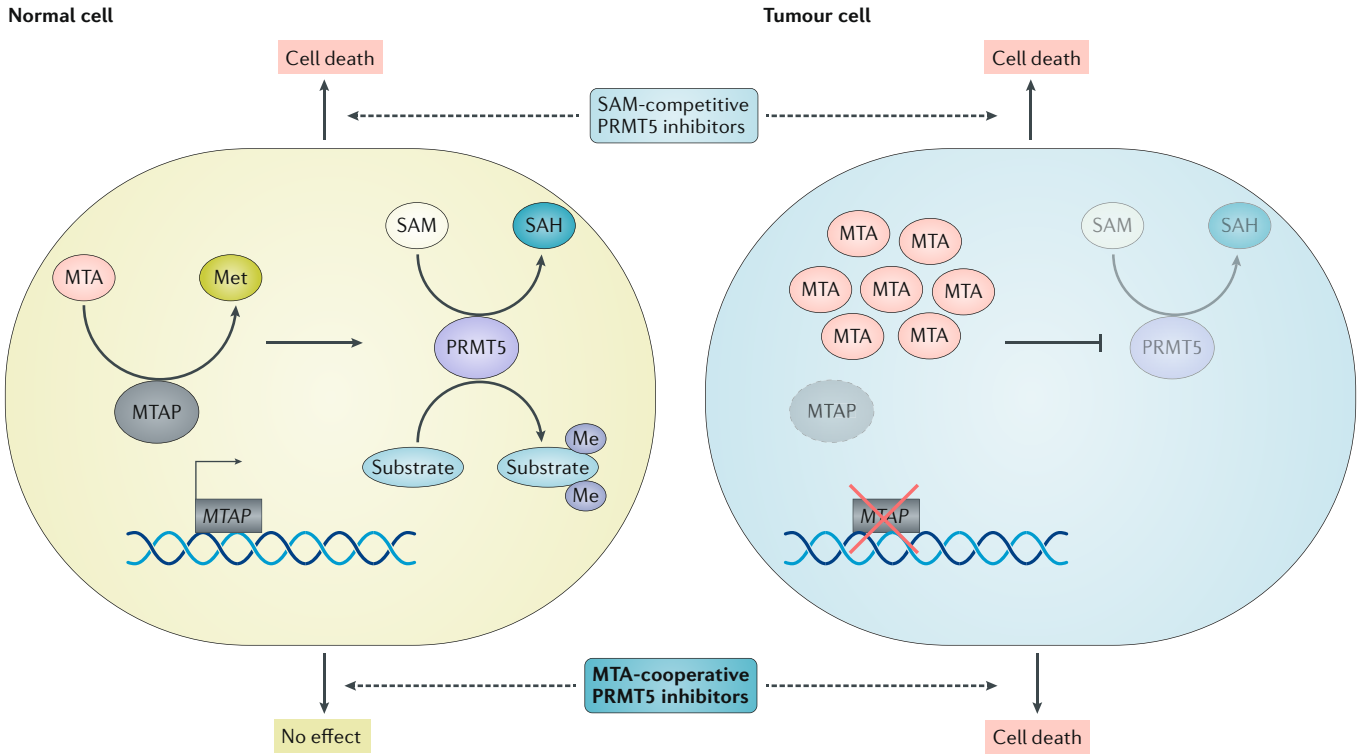

Fig. 2 | **PRMT5 and MTAP are a synthetic lethal pair.** Methylthioadenosine phosphorylase (MTAP) is frequently deleted in human tumours, causing its substrate methyl-5′-thioadenosine (MTA) to accumulate. MTA functions as a competitive inhibitor for the *S*-methyl-5′-thioadenosine phosphorylase (PRMT5)-activating cofactor *S*-adenosylmethionine (SAM), which provides a methyl donor group for client proteins. Therefore, MTA accumulation reduces, but does not eliminate, PRMT5 activity. PRMT5 inhibitors that leverage the accumulation of MTA (MTA-cooperative inhibitors) should drive selectivity for the MTAP-deleted tumours while sparing the wild-type normal cells. Existing PRMT5 inhibitors that are SAM cooperative do not leverage MTA accumulation, inhibit PRMT5 regardless of the genetic context and therefore are not selective for MTAP-deleted tumours. SAH, *S*-adenosyl-L-homocysteine.

fulfilled with the lack of success of these efforts. This is at least partly due to the lack of selective and potent pharmacologic reagents[136]. Furthermore, hypothesis-driven discovery of synthetic lethal DNA repair pathways is limited by the sheer volume of hypotheses to test with seven well-described unique DNA repair pathways and multiple genes within each.

Given the potentially large number of synthetic lethal interactions in the human genome, it is clear that discovery of additional synthetic lethal pairs amenable to drug discovery requires a functional genomic approach. To this end, a CRISPR library can be used to identify synthetic lethal 'hits' in cell line panels that share loss of a specific tumour suppressor gene matched as closely as possibly to cell lines that retain wild-type function of the gene of interest. Hits from these screens are potential drug targets, and the patient population expected to benefit from inhibiting the novel targets is defined by the genetic context being interrogated.

***MTAP* deletion and PRMT5 inhibition: synthetic lethality beyond PARP inhibitors.** One of the strongest and most prevalent synthetic lethal interactions discovered by Project Achilles and Project DRIVE is PRMT5 dependence in cells with *MTAP* deletions[72–74]. This dependency represents a subset of synthetic lethality termed 'collateral lethality'[137]. Collateral lethality occurs when a 'passenger' gene adjacent to a tumour suppressor gene is lost along with the 'driver' gene. In this case, *MTAP* is the passenger gene and is frequently co-deleted with the driver cyclin-dependent kinase inhibitor (*CDKN2A*, encoding p16-INK4)[72–74]. Several groups discovered that *MTAP*-null cancer cells have a marked dependency on PRMT5, an essential methyltransferase, making these cells much more susceptible to PRMT5 knockdown than those without *MTAP* deletion[72–74] (FIG. 2). This dependency occurs because *MTAP*-null cells accumulate high levels of the PRMT5 inhibitory cofactor *S*-methyl-5′-thioadenosine (MTA). As a result, PRMT5 is partially inhibited at the baseline in *MTAP*-null cells, and they are profoundly sensitive to further reduction of PRMT5 activity (for example, by genetic knockdown). This dependency provides the potential for a large therapeutic window for PRMT5 inhibitors in patients with *MTAP*-deleted tumours given that normal cells (without *MTAP* deletion) would be largely spared, limiting toxicity.

MTAP is deleted in approximately 15% of all human cancers, including more than 50% of glioblastomas and 25% of pancreatic cancers; thus, development of an effective therapy for *MTAP*-deleted tumours could have high patient impact. However, the effect of PRMT5 knockdown has not been recapitulated with existing PRMT5 inhibitors[72–74]. The lack of concordance between

Competitive inhibitors
Small molecules that compete with the substrates or cofactors when binding to the target enzyme, resulting in functional inhibition. By contrast, an uncompetitive inhibitor binds to an enzyme–substrate complex more tightly than to the enzyme alone, also resulting in functional inhibition.

genetic knockdown and pharmacologic inhibition in this setting could suggest that functional genomics may not be a good drug target discovery tool, when it is in fact due to the mechanism of action of existing inhibitors. PRMT5 has two cofactors: the activating cofactor *S*-adenosylmethionine (SAM) and the inhibitory cofactor MTA. All known PRMT5 inhibitors are either SAM-cooperative inhibitors (GlaxoSmithKline) or MTA-competitive inhibitors (Eli Lilly and Company, Johnson & Johnson). Because MTA accumulates in *MTAP*-deleted cancers to much higher levels than in normal cells, an MTA-cooperative and SAM-competitive PRMT5 inhibitor will be required to recapitulate the effect of *PRMT5* knockdown[73]. Such an inhibitor would increase the amount of inactive PRMT5 (bound to MTA) relative to active PRMT5 (bound to SAM), which would result in death of *MTAP*-deficient cells but not wild-type cells. Existing PRMT5 inhibitors do not act by this mechanism and therefore kill both *MTAP*-deleted cells and wild-type cells at similar exposures, resulting in toxicity with limited activity[73]. MTA-cooperative, SAM-competitive PRMT5 inhibitors are likely in development but not currently available. Instead, the first effort to exploit this synthetic lethal pair has been with MAT2A inhibitors, another component of the metabolic pathway that acts by reducing SAM levels. The first MAT2A inhibitor is now in clinical trials but limited efficacy data have been released[138].

Beyond synthetic lethal targets

Combining CRISPR-based screening with known targeted drugs. DNA sequencing provided the means to identify the first wave of genetic drug targets for specific cancer subtypes because they are 'marked' with fixed genetic alterations, and many of these genetically altered oncogenes have now been successfully drugged. As noted earlier, BRAF inhibitors and HER2 inhibitors are very active drugs in *BRAF*-mutant melanoma and *ERBB2*-amplified breast cancer, respectively. However, these inhibitors have limited activity, despite the presence of the same genetic alterations, in colon and gastric cancer. Thus, *BRAF* and *ERBB2* are context-dependent 'marked' oncogenes. We define 'unmarked oncogenes' as genes that are not genetically activated through mutation, amplification or translocation but are similarly important oncogenic drivers in specific genetic contexts. On the basis of the well-described concept of non-oncogene addition, which is driven by 'unmarked oncogenes', we postulate that there are many such oncogenes relevant to specific genetic contexts Many of these genes will be good drug targets but will require a functional genomic screening approach for discovery, as hypothesis-driven approaches are hampered by our limited understanding of the biology of complex systems and the reality that empiric approaches are not scalable. A modified synthetic lethal screening platform can be used to identify novel context-dependent unmarked oncogenes that may be good drug targets for single-agent or combination therapy.

This concept is exemplified by the finding that breast cancers that express oestrogen receptor (ER positive) are sensitive to a combination of inhibition of ER and inhibition of CDK4 and CDK6 (CDK4/6)[139–142], an active combination because of the crosstalk between ER and cell cycle signalling[143]. The discovery of this interaction came from profiling of a CDK4/6 inhibitor in a panel of 47 breast cancer cell lines and finding of significant growth inhibition clearly limited to the ER-positive cell lines in the panel. CDK4/6 inhibition was therefore tested in combination with several modulators of oestrogen blockade, including tamoxifen, and was found to be synergistic both preclinically and in patients[139,142]. As single agents, CDK4/6 inhibitors are not active in ER-positive breast cancer, and the combination of CDK4/6 inhibition and oestrogen blockade is not active in ER-negative breast cancer subtypes. Thus, *CDK4*/*CDK6* is a context-dependent 'unmarked' oncogene in the ER-positive breast cancer context. This hypothesis-driven approach led to an important clinical advance, but it is not scalable. Empiric approaches to combination discovery have been largely unsuccessful, and are prohibitively expensive if performed in clinical trials as is commonplace at present with immunologic agents, highlighting the potential value of a functional genomics strategy.

Novel drug combination discovery. One approach to discovering drug combinations is to uncover novel context-dependent unmarked oncogenes using a CRISPR library in combination with a targeted drug that is relevant to a specific genetic context. We define this as an 'anchor' screen, in which a targeted drug is the 'anchor' (FIG. 3). This approach was used by Bernards and colleagues in 2012 using shRNA-based screening (before CRISPR systems were widely available) to identify combination drug targets that would enhance the efficacy of the BRAF inhibitor vemurafenib in *BRAF*-mutant colon cancer[144]. Vemurafenib has a 50–60% overall response rate in melanoma[19,20] but only a 5% overall response rate in colon cancers[145,146] with activating *BRAF* mutations. With use of a focused library of shRNAs representing 518 kinases (the 'kinome'), a total of six colon cancer cell lines with or without $BRAF^{V600E}$ activating mutations were screened for genetic dependencies in the presence of vemurafenib. This drug anchor screen identified EGFR as a combination target with vemurafenib in this context[144]. This combination has since been shown to be clinically active, with an EGFR inhibitor doubling the response rate of BRAF inhibition alone in *BRAF*-mutant colon cancer, yet unfortunately remaining at a very modest 10% overall response rate[147]. The Bernards group also used a 298-gene 'phosphatome' shRNA library to identify PTPN11 inhibitors as another way to enhance the effect of BRAF inhibitors in this setting[148], and clinical trials to test this hypothesis are ongoing[149,150]. Garraway and colleagues also conducted a BRAF inhibitor anchor screen in a *BRAF*-mutant colon cancer cell line but instead used a larger, genome-scale shRNA library and discovered additional combination targets, although the conventionally druggable targets identified by this much larger screen were included in the smaller, more manageable kinome and phosphatome libraries[144,148,151]. Finally, a similar approach has been taken to identify combination partners for MEK inhibitors as several potent and selective MEK inhibitors are in clinical use, but they

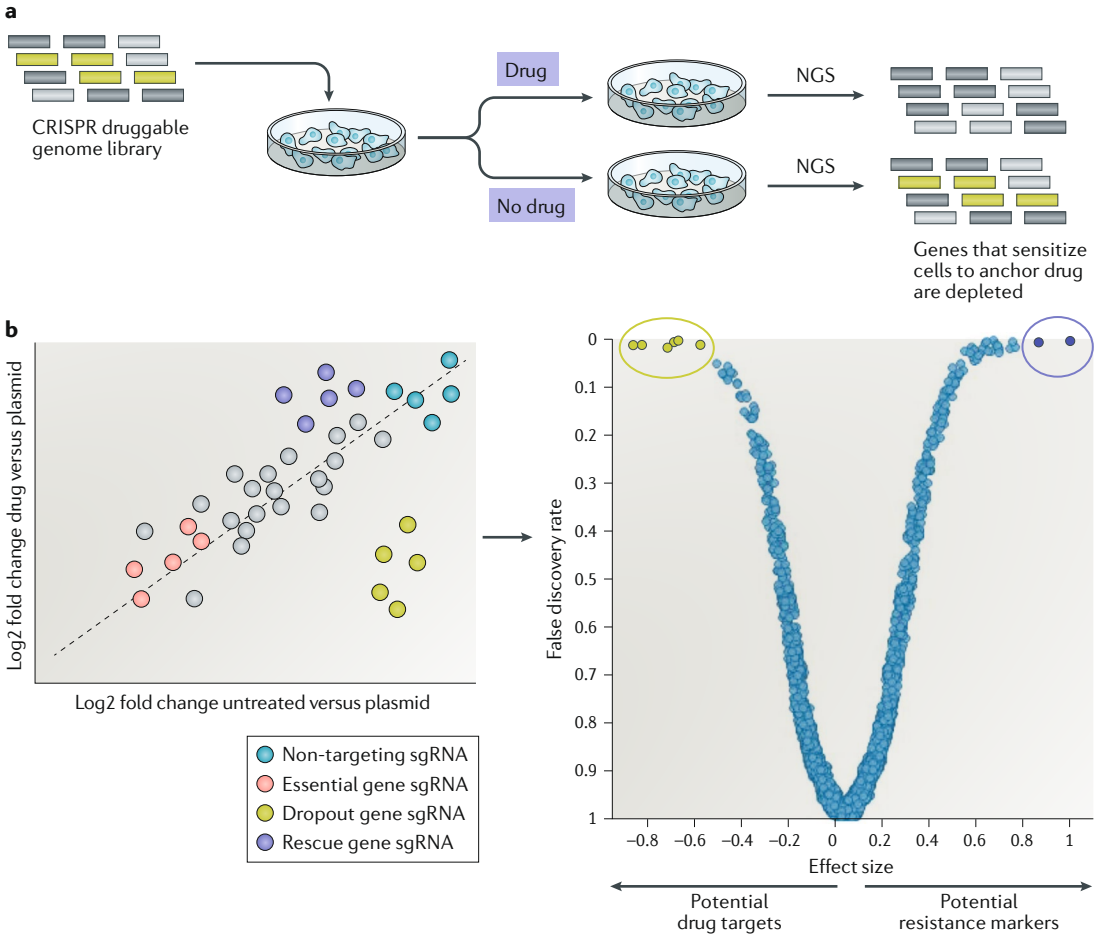

Fig. 3 | **Identifying novel combination targets using CRISPR screening. a** | Relevant cancer cell lines with or without a mutation of interest are infected with a CRISPR library and then passaged in the presence or absence of selective small-molecule inhibitors at their clinically relevant doses. At the end of the treatment period, cells are harvested, and the abundance of each guide RNA (gRNA) is evaluated using next-generation sequencing (NGS). Levels of gRNA are compared between drug treatment samples and control (no drug) samples to identify those gRNAs that are selectively depleted in the presence of the drug (green gRNAs). **b** | gRNA counts from NGS are quantified, then normalized and compared with the plasmid library. Genes that sensitize (which are potential drug targets in combination with the anchor drug) or rescue (which are markers of resistance to the anchor drug) cell viability to the anchor drug would selectively be depleted or enriched in the drug-treated group but not the control group (left panel). The results of this effect are statistically quantified using MAGeCK[198] to identify both potential drug targets (dropouts) and resistance marker (rescue) genes (right panel). sgRNA, single-guide RNA.

are minimally active in *KRAS*-mutant NSCLC[152,153]. An shRNA-based kinome anchor screen identified ERBB3 blockade as an approach for enhancing the effect of MEK inhibition in this setting, a hypothesis that remains to be tested in clinical studies[130]. These anchor screens, although informative, can now be enhanced by use of CRISPR technologies as the genetic screening tool. As a result, anchor screens can be widely applied to identify drug combinations with either known or novel targets that will be required to start seeing sustained, complete responses in patients with metastatic solid tumours.

Screening for non-cell-autonomous targets: immune evasion context. The classic definition of synthetic lethality is cell autonomous, but a synthetic lethal approach can be adapted to identify druggable targets that do not kill cancer cells directly, but rather attract immune cells to destroy them. As has been well described, the accumulation of genetic alterations in cancer cells creates neoantigens, which should be recognized by the immune system as 'foreign' and trigger immune destruction of nascent cancers[154,155]. However, all cancers that become clinically relevant have, by definition, escaped immune destruction. A wide variety of immune evasion mechanisms have been postulated, including immune editing, T cell exhaustion and an inhibitory microenvironment[156], but important drivers of immune evasion must emanate at least in part from the cancer cell itself. However, the genetics of tumour-intrinsic immune evasion have only recently begun to be described[155,157,158] and no drug targets with the potential to reverse this hallmark of cancer have yet been discovered.

In 2016, Ribas and colleagues reported that loss-of-function mutations in *JAK1* enhance immune evasion and conferred anti-PD1 resistance in a patient treated with a checkpoint inhibitor[158]. This study provided the

first evidence that a tumour cell can evade immune pressure by evolving intrinsic genetic changes. Additional recurring loss-of-function mutations were subsequently identified in genes encoding other components of the antigen presentation machinery, such as components of the class I major histocompatibility complex HLA and *B2M*, further supporting this observation[159–161]. Emerging data also indicate that many well-studied oncogenic drivers and tumour suppressor genes may play prominent parts in immune evasion. One example of a putative tumour-intrinsic immune evasion gene is the protein kinase gene *LKB1* (also known as *STK11*), a tumour suppressor gene inactivated in ~20% of NSCLC[97]. Loss of function of *LKB1* has been postulated to drive tumorigenesis through activation of the mechanistic target of rapamycin pathway in a cancer cell-autonomous manner[162]. However, *LKB1* loss also results in accumulation of neutrophils with T cell suppressive effects and an increase in the levels of tumour-promoting cytokines[163]. A retrospective analysis of tumours from patients who did not respond to treatment with PD1 inhibitors correlated *LKB1* loss with reduced PD-L1 expression[164], further suggesting that *LKB1* is a bona fide suppressor of immune evasion, which may be one of its primary functions. Other genetic alterations linked to immune evasion include *MYCN* amplification, which limits T cell infiltration in neuroblastoma through downregulation of the interferon response[165], *CASP8* loss of function, which rescues cancer cells from T cell-mediated lethality by blocking the tumour necrosis factor pathway[166] and *PTEN* loss of function, which promotes resistance to T cell killing by increasing production of immunosuppressive cytokines[167]. Thus, a systematic functional genomic evaluation of all known cancer genes is warranted to then allow identification of drug targets that reverse immune evasion signalling. In this setting, the immune evasion gene (such as *LKB1*) provides the context in which a novel drug target (to be determined) functions as the patient selection biomarker for clinical trial development.

Approved drugs that target the immune cell checkpoints PD1, PD-L1 and CTLA4 are now widely used as anticancer therapy, but these medicines are directed towards and activate T cells as opposed to targeting tumour-intrinsic immune evasion mechanisms. Building on the success of the current treatment paradigm, the discovery of novel immuno-oncology targets and development efforts are almost exclusively focused on host immunity, such as modulating T cells, natural killer cells, macrophages and the tumour microenvironment. Therefore, a novel approach that targets tumour-intrinsic immune evasion mechanisms (likely in combination with checkpoint inhibitors) will have several advantages. First, it will open a largely unexplored target space. Second, targeting tumour-intrinsic mechanisms will direct immune cells to the tumour specifically and may limit the systemic autoimmune toxicity that is the primary toxic effect of checkpoint inhibitors[168]. Finally, the genetic context in which specific immune evasion targets are active will define patient selection biomarkers that have been elusive for checkpoint inhibitor therapy. Such drugs could be used in combination with reagents stimulating host immunity, such as checkpoint inhibitors. In addition, single-agent activity may be possible in cases in which active T cell infiltration has already occurred in the tumour environment and in which the tumour cell-driven immune evasion mechanism is the key gatekeeping event for preventing immune eradication. This approach has the potential to discover novel medicines for cancers that are insensitive to immune checkpoint inhibition and have the advantage of defining a genetic patient selection strategy for clinical development.

Discovering tumour-intrinsic immune evasion targets requires a two-step process: first, a genetic context that confers immune evasion is identified and, second, the drug targets that reverse such a phenotype are identified (FIG. 4). In the first step, a synthetic lethal-based CRISPR screen can be applied by changing the readout from growth rate alterations or cell death to immune cell-mediated lethality and using in vivo screening approaches. Providing a strong proof of principle, an in vivo CRISPR screen that evaluated 2,700 candidate genes was recently reported in B16 syngeneic mice treated with PD1 checkpoint blockade[169]. When the tumours were collected and sequenced, genes driving immune evasion and immune sensitization were identified by comparison of their relative abundance in tumours grown under increasing immune pressure. Known immune evasion genetic contexts, such as *JAK1* and *B2M* loss of function, scored highly in this study. However, to expand these findings, studies that comprehensively identify the genetic contexts that drive immune evasion are needed. These context discovery screens will define the genetic contexts in which to conduct target discovery screens, the second step in tumour-intrinsic immune evasion target discovery.

Once an immune evasion genetic context has been discovered, target discovery screens can be performed either in vivo or in vitro. In vivo models are more reflective of the human immune system, whereas in vitro screens are more reflective of histology-specific human cancer genetics. In vivo screens have the advantage of an intact immune system, but require special consideration. There are limited numbers of syngeneic mouse models, and those that exist typically have chemically induced tumours and therefore a mutation spectrum that is not reflective of corresponding human cancers. In addition, the throughput of in vivo CRISPR screening is limited by the library size (generally fewer than 4,000 sgRNAs) and the number of cells that can be implanted in each mouse. By contrast, there are many genetically engineered murine models designed to study human cancer genetics, but they are mostly non-immunogenic and are therefore less useful for immune evasion context and target discovery.

When the mechanism of gene-specific immune evasion is known or postulated, in vitro screening with a relevant immune readout such as PD-L1 expression may be the most efficient approach for identifying drug targets. For example, *JAK1* loss of function mediates immune evasion through suppression of interferon signalling[170,171]. Therefore, a target discovery screen in human cancer cell lines that harbour a *JAK1* inactivating

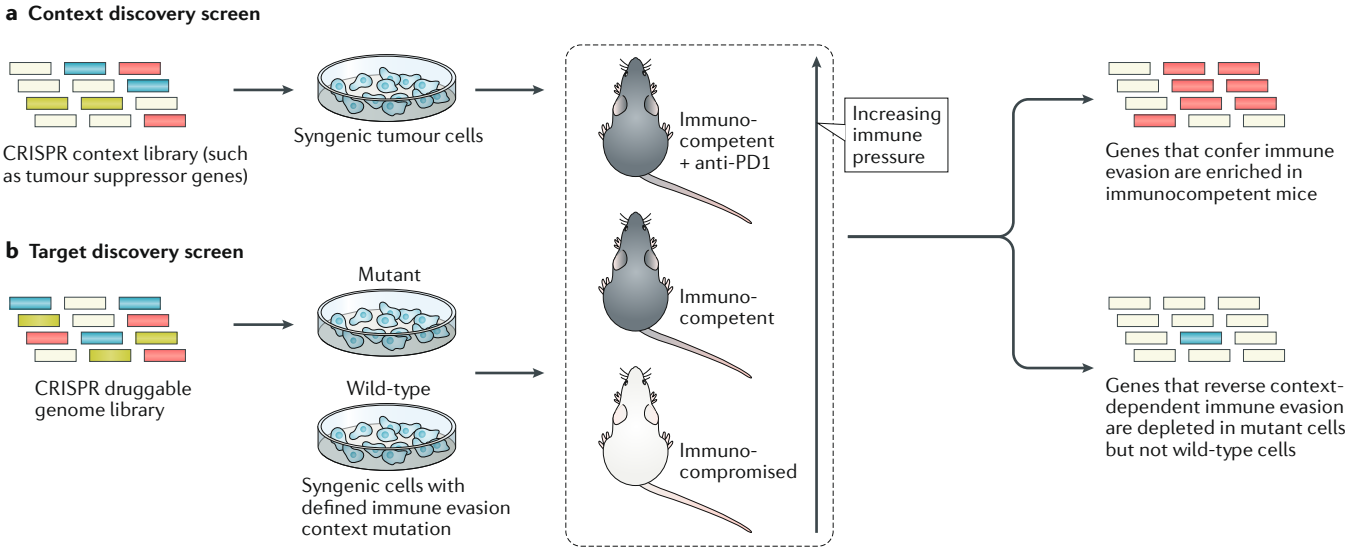

Fig. 4 | **Identifying synthetic lethal drug targets that reverse tumour-intrinsic immune evasion. a** | Syngeneic mouse models with known responsiveness to immune therapies, such as MC38 and CT26, can be used to screen mice for genes that drive immune evasion when inactivated (immune evasion genes). A 'context discovery' CRISPR library that consists of candidate immune evasion genes can then be introduced into the tumour models as a pool using lentiviral infection. Infected cells are subsequently implanted into different mouse strains that are either immunocompromised or immunocompetent. Immune pressure in the immunocompetent mice can be further enhanced by activating T cells with an anti-programmed cell death 1 (anti-PD1) antibody. Tumours are then allowed to grow, and at the time of tumour harvesting, cells with immune evasion gene loss will survive best in models with the highest immune pressure. Those genes can be identified using next-generation sequencing to measure the relative abundance of guide RNA. **b** | For the discovery of drug targets that reverse immune evasion driven in a specific genetic context, genetically engineered cell lines expressing genes that confer an immune evasion genetic context (mutant) and wild-type cells are infected with a druggable genome CRISPR library using the schema described above to supply immune pressure. Novel drug targets can be identified by identifying those guide RNAs depleted in the presence of immune pressure in mutant cells but not wild-type cells.

mutation could measure PD-L1 induction following interferon stimulation. Additional in vitro screening approaches may include tumour cell and T cell co-cultures, such as the ovalbumin mouse model, in which T cells are engineered to express the relevant T cell receptor for ovalbumin recognition, and the tumour cells are modified to express chicken ovalbumin antigen[166]. Such systems can be used to interrogate the phenotype of T cell-mediated killing directly and could bridge in vivo and in vitro approaches.

As the underlying cancer genetics that drive immune evasion become better understood, the tools of synthetic lethal-based target discovery can be used to discover novel tumour-intrinsic drug targets that reverse immune evasion. Context-dependent, tumour-intrinsic, immune evasion target discovery screens can be conducted using paired isogenic models engineered with the immune evasion gene loss (such as *JAK1* loss of function) and a wild-type control. Potential drug targets are those genes that reverse the immune evasion phenotype when knocked out. These targets have the potential to overcome immune checkpoint inhibitor resistance and thereby address a significant unmet medical need.

Future directions

We are reaching the limits of sequence-based genetic target discovery for cancer but there are compelling reasons to believe that many cancer drug targets are still to be identified. Recent analysis of a genome-scale CRISPR-based screen in a large panel of cancer cell lines provides direct evidence that this number is likely in the hundreds, but the large majority will be context specific[92]. Applying the genetic concept of synthetic lethality with both its classic definition and with some conceptual modifications to identify these drug targets, and the genetic contexts (and therefore patients) in which they will be effective therapies, represents a transformative opportunity for patients, and the tools with which to do this are becoming ever more powerful. Continuously evolving CRISPR technology, including single-cell techniques, holds the promise of addressing the tumour heterogeneity involved in primary drug resistance and the genetic evolution that drives secondary resistance. Combination CRISPR vectors will simplify the search for effective, context-specific drug combinations, and base-editing techniques are streamlining the construction of the tools needed for target validation. Finally, the continued refinement of technologies that will make in vivo CRISPR screening as scalable as in vitro approaches is enabling the integration of cancer genetics and immuno-oncology and driving our understanding of the genetic basis of immune evasion. Enhanced by these new tools, CRISPR-based functional genomic screening can be applied in increasingly unique ways to address the loss of tumour suppressor genes, unmarked oncogenes and non-cell-autonomous pathways, and will yield the next wave of effective targeted therapies.

New technologies that expand the number of targets that are considered druggable have the potential to have a great impact on drug discovery. Targeted protein degradation is one such example that could markedly expand the number of druggable targets. Despite the great promise of these approaches, there remain many challenges in moving from the novel target discovery that is now within our grasp to medicines that are clinically effective. The large majority of novel targets will be intracellular proteins that require a small-molecule inhibitor for clinical activity. Under the best of circumstances, 5 years from target discovery to a clinical proof of concept is considered 'fast', so time alone is a hurdle that is difficult to avoid with current drug discovery technology. In addition to the frustratingly long timelines for drug discovery, the impact of context dependency means that focused functional genomic discovery approaches need to be applied to many cancer subsets, further compounding the time needed to bring truly transformative combinations to a wide spectrum of patients. Finally, evolving drug resistance is inevitable until complete tumour ablation is achieved, likely requiring a combination of targeted therapy and immunotherapy. Thus, understanding resistance mechanisms and the application of this approach in those settings will also be essential. Nonetheless, CRISPR, with all of its current and future applications, is a tool that has opened the door to an enormous range of possibilities. Many people with cancer have been cured in our lifetimes, an achievement not thought possible when synthetic lethality was first discovered in fruit flies, and many more people will be cured as a result of the work now made possible by applying the concept of synthetic lethality with the genome editing power of CRISPR technology.

Published online: 11 November 2019

1. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
2. Lander, E. S. et al. Initial sequencing and analysis of the human genome. *Nature* **409**, 860–921 (2001).
3. Venter, J. C. et al. The sequence of the human genome. *Science* **291**, 1304–1351 (2001).
4. Hoadley, K. A. et al. Cell-of-origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer. *Cell* **173**, 291–304.e6 (2018).
5. Tate, J. G. et al. COSMIC: the catalogue of somatic mutations in cancer. *Nucleic Acids Res.* **47**, D941–D947 (2019).
6. International Cancer Genome Consortium. International network of cancer genome projects. *Nature* **464**, 993–998 (2010).
7. Cancer Genome Atlas Research Network et al. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat. Genet.* **45**, 1113–1120 (2013).
8. Hochhaus, A. et al. Long-term outcomes of imatinib treatment for chronic myeloid leukemia. *N. Engl. J. Med.* **376**, 917–927 (2017).
9. Druker, B. J. et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N. Engl. J. Med.* **355**, 2408–2417 (2006).
10. Kantarjian, H. et al. Improved survival in chronic myeloid leukemia since the introduction of imatinib therapy: a single-institution historical experience. *Blood* **119**, 1981–1987 (2012).
11. Roy, L. et al. Survival advantage from imatinib compared with the combination interferon-α plus cytarabine in chronic-phase chronic myelogenous leukemia: historical comparison between two phase 3 trials. *Blood* **108**, 1478–1484 (2006).
12. Verweij, J. et al. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. *Lancet* **364**, 1127–1134 (2004).
13. Blanke, C. D. et al. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. *J. Clin. Oncol.* **26**, 626–632 (2008).
14. Blanke, C. D. et al. Long-term results from a randomized phase II trial of standard- versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumors expressing KIT. *J. Clin. Oncol.* **26**, 620–625 (2008).
15. Demetri, G. D. et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N. Engl. J. Med.* **347**, 472–480 (2002).
16. Bollag, G. et al. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature* **467**, 596–599 (2010).
17. Rheault, T. R. et al. Discovery of dabrafenib: a selective inhibitor of Raf kinases with antitumor activity against B-Raf-driven tumors. *ACS Med. Chem. Lett.* **4**, 358–362 (2013).
18. Delord, J. P. et al. Phase I dose-escalation and -expansion study of the BRAF inhibitor encorafenib (LGX818) in metastatic BRAF-mutant melanoma. *Clin. Cancer Res.* **23**, 5339–5348 (2017).
19. Chapman, P. B. et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N. Engl. J. Med.* **364**, 2507–2516 (2011).
20. Hauschild, A. et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* **380**, 358–365 (2012).
21. Larkin, J. et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N. Engl. J. Med.* **371**, 1867–1876 (2014).
22. Robert, C. et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. *N. Engl. J. Med.* **372**, 30–39 (2015).
23. Dummer, R. et al. Overall survival in patients with BRAF-mutant melanoma receiving encorafenib plus binimetinib versus vemurafenib or encorafenib (COLUMBUS): a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol.* **19**, 1315–1327 (2018).
24. Slamon, D. J. et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* **344**, 783–792 (2001).
25. Baselga, J. et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N. Engl. J. Med.* **366**, 109–119 (2012).
26. Mok, T. S. et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N. Engl. J. Med.* **361**, 947–957 (2009).
27. Maemondo, M. et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N. Engl. J. Med.* **362**, 2380–2388 (2010).
28. Mitsudomi, T. et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol.* **11**, 121–128 (2010).
29. Zhou, C. et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol.* **12**, 735–742 (2011).
30. Rosell, R. et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol.* **13**, 239–246 (2012).
31. Sequist, L. V. et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J. Clin. Oncol.* **31**, 3327–3334 (2013).
32. Soria, J. C. et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N. Engl. J. Med.* **378**, 113–125 (2018).
33. Solomon, B. J. et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N. Engl. J. Med.* **371**, 2167–2177 (2014).
34. Shaw, A. T. et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N. Engl. J. Med.* **368**, 2385–2394 (2013).
35. Shaw, A. T. & Engelman, J. A. Ceritinib in ALK-rearranged non-small-cell lung cancer. *N. Engl. J. Med.* **370**, 2537–2539 (2014).
36. Sawyers, C. Targeted cancer therapy. *Nature* **432**, 294–297 (2004).
37. Lawrence, M. S. et al. Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature* **505**, 495–501 (2014).
This landmark study integrates large-scale next-generation whole-exome sequencing data from human tumours to define the landscape of tumour suppressor genes and oncogenes across 21 different cancer types.
38. Bailey, M. H. et al. Comprehensive characterization of cancer driver genes and mutations. *Cell* **173**, 371–385 e318 (2018).
39. Beroukhim, R. et al. The landscape of somatic copy-number alteration across human cancers. *Nature* **463**, 899–905 (2010).
40. Churcher, I. Protac-induced protein degradation in drug discovery: breaking the rules or just making new ones? *J. Med. Chem.* **61**, 444–452 (2018).
41. Lai, A. C. & Crews, C. M. Induced protein degradation: an emerging drug discovery paradigm. *Nat. Rev. Drug Discov.* **16**, 101–114 (2017).
42. Robert, C. et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N. Engl. J. Med.* **372**, 320–330 (2015).
43. Schachter, J. et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). *Lancet* **390**, 1853–1862 (2017).
44. Weber, J. S. et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol.* **16**, 375–384 (2015).
45. Motzer, R. J. et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N. Engl. J. Med.* **373**, 1803–1813 (2015).
46. Brahmer, J. et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N. Engl. J. Med.* **373**, 123–135 (2015).
47. Borghaei, H. et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N. Engl. J. Med.* **373**, 1627–1639 (2015).
48. Reck, M. et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N. Engl. J. Med.* **375**, 1823–1833 (2016).
49. Langer, C. J. et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *Lancet Oncol.* **17**, 1497–1508 (2016).
50. Sun, C., Mezzadra, R. & Schumacher, T. N. Regulation and function of the PD-L1 checkpoint. *Immunity* **48**, 434–452 (2018).
51. Hodi, F. S. et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* **363**, 711–723 (2010).
52. Larkin, J. et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N. Engl. J. Med.* **373**, 23–34 (2015).

53. Tang, J. et al. The clinical trial landscape for PD1/PDL1 immune checkpoint inhibitors. *Nat. Rev. Drug Discov.* **17**, 854–855 (2018).
54. Weinstein, I. B. Addiction to oncogenes–the Achilles heal of cancer. *Science* **297**, 63–64 (2002).
55. Solimini, N. L., Luo, J. & Elledge, S. J. Non-oncogene addiction and the stress phenotype of cancer cells. *Cell* **130**, 986–988 (2007).
56. Dobzhansky, T. Genetics of natural populations; recombination and variability in populations of Drosophila pseudoobscura. *Genetics* **31**, 269–290 (1946).
57. Hartwell, L. H., Szankasi, P., Roberts, C. J., Murray, A. W. & Friend, S. H. Integrating genetic approaches into the discovery of anticancer drugs. *Science* **278**, 1064–1068 (1997).
These authors proposed, for the first time, applying the genetic principle of synthetic lethality using yeast genetic screens to identify novel vulnerabilities in cancer cells based on defined genetic defects.
58. Kaelin, W. G. Jr Choosing anticancer drug targets in the postgenomic era. *J. Clin. Invest.* **104**, 1503–1506 (1999).
59. Kaelin, W. G. Jr The concept of synthetic lethality in the context of anticancer therapy. *Nat. Rev. Cancer* **5**, 689–698 (2005).
60. Sturtevant, A. H. A highly specific complementary lethal system in *Drosophila melanogaster*. *Genetics* **41**, 118–123 (1956).
61. Lucchesi, J. C. Synthetic lethality and semi-lethality among functionally related mutants of *Drosophila melanogaster*. *Genet.* **59**, 37–44 (1968).
62. Bridges, C. B. The origin of variations in sexual and sex-limited characters. *Am. Nat.* **56**, 51–63 (1922).
63. Bender, A. & Pringle, J. R. Use of a screen for synthetic lethal and multicopy suppressee mutants to identify two new genes involved in morphogenesis in Saccharomyces cerevisiae. *Mol. Cell Biol.* **11**, 1295–1305 (1991).
64. Kaiser, C. A. & Schekman, R. Distinct sets of SEC genes govern transport vesicle formation and fusion early in the secretory pathway. *Cell* **61**, 723–733 (1990).
65. Hennessy, K. M., Lee, A., Chen, E. & Botstein, D. A group of interacting yeast DNA replication genes. *Genes Dev.* **5**, 958–969 (1991).
66. Mullenders, J. & Bernards, R. Loss-of-function genetic screens as a tool to improve the diagnosis and treatment of cancer. *Oncogene* **28**, 4409–4420 (2009).
67. Bommi-Reddy, A. et al. Kinase requirements in human cells: III. Altered kinase requirements in VHL–/– cancer cells detected in a pilot synthetic lethal screen. *Proc. Natl Acad. Sci. USA* **105**, 16484–16489 (2008).
68. Hoffman, G. R. et al. Functional epigenetics approach identifies BRM/SMARCA2 as a critical synthetic lethal target in BRG1-deficient cancers. *Proc. Natl Acad. Sci. USA* **111**, 3128–3133 (2014).
This is the first report to demonstrate the synthetic lethal interaction between *SMARCA2* and *SMARCA4*.
69. Wilson, B. G. et al. Residual complexes containing SMARCA2 (BRM) underlie the oncogenic drive of SMARCA4 (BRG1) mutation. *Mol. Cell Biol.* **34**, 1136–1144 (2014).
70. Oike, T. et al. A synthetic lethality-based strategy to treat cancers harboring a genetic deficiency in the chromatin remodeling factor BRG1. *Cancer Res.* **73**, 5508–5518 (2013).
71. Helming, K. C. et al. ARID1B is a specific vulnerability in ARID1A-mutant cancers. *Nat. Med.* **20**, 251–254 (2014).
72. Kryukov, G. V. et al. MTAP deletion confers enhanced dependency on the PRMT5 arginine methyltransferase in cancer cells. *Science* **351**, 1214–1218 (2016).
73. Mavrakis, K. J. et al. Disordered methionine metabolism in MTAP/CDKN2A-deleted cancers leads to dependence on PRMT5. *Science* **351**, 1208–1213 (2016).
References 72 and 73 are the first to demonstrate the synthetic lethal interaction between PRMT5 and *MTAP* deletion. *MTAP* is lost as a passenger owing to proximity to *CDKN2A*; therefore, this is one of the first examples of collateral lethality.
74. Marjon, K. et al. MTAP deletions in cancer create vulnerability to targeting of the MAT2A/PRMT5/RIOK1 axis. *Cell Rep.* **15**, 574–587 (2016).
75. Farmer, H. et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434**, 917–921 (2005).
76. Lord, C. J. & Ashworth, A. PARP inhibitors: synthetic lethality in the clinic. *Science* **355**, 1152–1158 (2017).
77. Ashworth, A. A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. *J. Clin. Oncol.* **26**, 3785–3790 (2008).
78. Fong, P. C. et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N. Engl. J. Med.* **361**, 123–134 (2009).
79. Bryant, H. E. et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* **434**, 913–917 (2005).
This article describes the discovery of the PARP–BRCA2 synthetic lethal relationship using a pharmacologic PARP inhibitor and isogenic mouse embryo fibrobast cell lines.
80. Murai, J. et al. Trapping of PARP1 and PARP2 by clinical PARP inhibitors. *Cancer Res.* **72**, 5588–5599 (2012).
81. Hopkins, T. A. et al. Mechanistic dissection of PARP1 trapping and the impact on in vivo tolerability and efficacy of PARP inhibitors. *Mol. Cancer Res.* **13**, 1465–1477 (2015).
82. Hopkins, T. A. et al. PARP1 Trapping by PARP inhibitors drives cytotoxicity in both cancer cells and healthy bone marrow. *Mol. Cancer Res.* **17**, 409–419 (2019).
83. Smith, I. et al. Evaluation of RNAi and CRISPR technologies by large-scale gene expression profiling in the connectivity map. *PLOS Biol.* **15**, e2003213 (2017).
84. Morgens, D. W., Deans, R. M., Li, A. & Bassik, M. C. Systematic comparison of CRISPR/Cas9 and RNAi screens for essential genes. *Nat. Biotechnol.* **4**, 634–636 (2016).
85. Evers, B. et al. CRISPR knockout screening outperforms shRNA and CRISPRi in identifying essential genes. *Nat. Biotechnol.* **34**, 631–633 (2016).
86. Barretina, J. et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* **483**, 603–607 (2012).
87. McDonald, E. R. 3rd et al. Project DRIVE: a compendium of cancer dependencies and synthetic lethal relationships uncovered by large-scale, deep RNAi screening. *Cell* **170**, 577–592.e10 (2017).
This article from Novartis describes the results of a large-scale shRNA-based functional genomic screen in an extensive panel of human cancer cell lines. This group used the integrated genomic and pathway data to systemically interrogate synthetic lethal and other dependency relationships across multiple histologies.
88. Cowley, G. S. et al. Parallel genome-scale loss of function screens in 216 cancer cell lines for the identification of context-specific genetic dependencies. *Sci. Data* **1**, 140035 (2014).
89. Tsherniak, A. et al. Defining a cancer dependency map. *Cell* **170**, 564–576.e16 (2017).
This article reports the results of a second shRNA-based functional genomic screen designed to identify genetic dependencies in approximately 500 human cancer cell lines. An informatics tool developed to eliminate false positive data from seed effects — and therefore to reduce the impact of off-target gene silencing — is included in this report.
90. Meyers, R. M. et al. Computational correction of copy number effect improves specificity of CRISPR-Cas9 essentiality screens in cancer cells. *Nat. Genet.* **49**, 1779–1784 (2017).
91. Aguirre, A. J. et al. Genomic copy number dictates a gene-independent cell response to CRISPR/Cas9 targeting. *Cancer Discov.* **6**, 914–929 (2016).
92. Behan, F. M. et al. Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. *Nature* **568**, 511–516 (2019).
This report from the Sanger Institute describes the results of a genome-wide CRISPR-based screen in a diverse human cancer cell panel. The analysis of the data was specifically designed to identify potential therapeutic targets and associated genetic alterations that could be used for patient selection in clinical studies.
93. Chan, E. M. et al. WRN helicase is a synthetic lethal target in microsatellite unstable cancers. *Nature* **568**, 551–556 (2019).
This article describes the discovery that the helicase WRN has a synthetic lethal interaction with microsatellite instability in colon cancer. Additional data suggest this effect will be histology agnostic.
94. Lieb, S. et al. Werner syndrome helicase is a selective vulnerability of microsatellite instability-high tumor cells. *eLife* **8**, e43333 (2019).
95. Kategaya, L., Perumal, S. K., Hager, J. H. & Belmont, L. D. Werner syndrome helicase is required for the survival of cancer cells with microsatellite instability. *iScience* **13**, 488–497 (2019).
96. Gillison, M. L., Chaturvedi, A. K., Anderson, W. F. & Fakhry, C. Epidemiology of human papillomavirus-positive head and neck squamous cell carcinoma. *J. Clin. Oncol.* **33**, 3235–3242 (2015).
97. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature* **511**, 543–550 (2014).
98. Kwak, E. L. et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N. Engl. J. Med.* **363**, 1693–1703 (2010).
99. Price, C. et al. Genome-wide interrogation of human cancers identifies EGLN1 dependency in clear cell ovarian cancers. *Cancer Res.* **79**, 2564–2579 (2019).
100. Kaelin, W. G. Jr & Ratcliffe, P. J. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol. Cell* **30**, 393–402 (2008).
101. Briggs, K. J., Min, C., Zhang, H. & Huang, A. Abstract 2892: EGLN1 is a synthetic lethal target in *ARID1A*-mutant ovarian cancer. *J. Cancer Res.* **78**, 2892–2892 (2018).
102. Jones, S. et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. *Science* **330**, 228–231 (2010).
103. Wiegand, K. C. et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. *N. Engl. J. Med.* **363**, 1532–1543 (2010).
104. Arana, M. E. et al. synthesis by human DNA polymerase theta. *Nucleic Acids Res.* **36**, 3847–3856 (2008).
105. Chan, S. H., Yu, A. M. & McVey, M. Dual roles for DNA polymerase theta in alternative end-joining repair of double-strand breaks in Drosophila. *PLOS Genet.* **6**, e1001005 (2010).
106. Roerink, S. F., van Schendel, R. & Tijsterman, M. Polymerase theta-mediated end joining of replication-associated DNA breaks in C. elegans. *Genome Res.* **24**, 954–962 (2014).
107. Kent, T., Chandramouly, G., McDevitt, S. M., Ozdemir, A. Y. & Pomerantz, R. T. Mechanism of microhomology-mediated end-joining promoted by human DNA polymerase theta. *Nat. Struct. Mol. Biol.* **22**, 230–237 (2015).
108. Mateos-Gomez, P. A. et al. Mammalian polymerase theta promotes alternative NHEJ and suppresses recombination. *Nature* **518**, 254–257 (2015).
109. Ceccaldi, R. et al. Homologous-recombination-deficient tumours are dependent on Poltheta-mediated repair. *Nature* **518**, 258–262 (2015).
110. Mengwasser, K. E. et al. Genetic screens reveal FEN1 and APEX2 as BRCA2 synthetic lethal targets. *Mol. Cell* **73**, 885–899.e6 (2019).
111. Nichols, R. J. et al. RAS nucleotide cycling underlies the SHP2 phosphatase dependence of mutant BRAF-, NF1- and RAS-driven cancers. *Nat. Cell Biol.* **20**, 1064–1073 (2018).
112. Chen, Y. N. et al. Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases. *Nature* **535**, 148–152 (2016).
113. Jinek, M. et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**, 816–821 (2012).
114. Cong, L. et al. Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819–823 (2013).
115. Mali, P. et al. RNA-guided human genome engineering via Cas9. *Science* **339**, 823–826 (2013).
116. Jinek, M. et al. RNA-programmed genome editing in human cells. *eLife* **2**, e00471 (2013).
117. Grimm, S. The art and design of genetic screens: mammalian culture cells. *Nat. Rev. Genet.* **5**, 179–189 (2004).
118. Boutros, M. & Ahringer, J. The art and design of genetic screens: RNA interference. *Nat. Rev. Genet.* **9**, 554–566 (2008).
119. Birmingham, A. et al. 3' UTR seed matches, but not overall identity, are associated with RNAi off-targets. *Nat. Methods* **3**, 199–204 (2006).
120. Buehler, E. et al. siRNA off-target effects in genome-wide screens identify signaling pathway members. *Sci. Rep.* **2**, 428 (2012).
121. Jackson, A. L. & Linsley, P. S. Noise amidst the silence: off-target effects of siRNAs? *Trends Genet.* **20**, 521–524 (2004).
122. Kampmann, M. CRISPRi and CRISPRa screens in mammalian cells for precision biology and medicine. *ACS Chem. Biol.* **13**, 406–416 (2018).
123. Shalem, O. et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* **343**, 84–87 (2014).
124. Wang, T., Wei, J. J., Sabatini, D. M. & Lander, E. S. Genetic screens in human cells using the CRISPR-Cas9 system. *Science* **343**, 80–84 (2014).

125. Koonin, E. V., Makarova, K. S. & Zhang, F. Diversity, classification and evolution of CRISPR-Cas systems. *Curr. Opin. Microbiol.* **37**, 67–78 (2017).
126. Shmakov, S. et al. Diversity and evolution of class 2 CRISPR-Cas systems. *Nat. Rev. Microbiol.* **15**, 169–182 (2017).
127. Ishino, Y., Krupovic, M. & Forterre, P. History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. *J. Bacteriol.* **200**, e00580-17 (2018).
128. Zetsche, B. et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell* **163**, 759–771 (2015).
These authors discover and develop the Cpf1 system as an alternative gene-editing approach to CRISPR–Cas9 and demonstrate the enhanced functionality of this system in combo-CRISPR screens using this system due to a markedly reduced recombination frequency.
129. Fonfara, I., Richter, H., Bratovic, M., Le Rhun, A. & Charpentier, E. The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. *Nature* **532**, 517–521 (2016).
130. Sun, C. et al. Intrinsic resistance to MEK inhibition in KRAS mutant lung and colon cancer through transcriptional induction of ERBB3. *Cell Rep.* **7**, 86–93 (2014).
131. Hopkins, A. L. & Groom, C. R. The druggable genome. *Nat. Rev. Drug Discov.* **1**, 727–730 (2002).
132. Griffith, M. et al. DGIdb: mining the druggable genome. *Nat. Methods* **10**, 1209–1210 (2013).
133. Santos, R. et al. A comprehensive map of molecular drug targets. *Nat. Rev. Drug Discov.* **16**, 19–34 (2016).
134. Duggan, S. P. et al. siRNA library screening identifies a druggable immune-signature driving esophageal adenocarcinoma cell growth. *Cell Mol. Gastroenterol. Hepatol.* **5**, 569–590 (2018).
135. Baratta, M. G. et al. An in-tumor genetic screen reveals that the BET bromodomain protein, BRD4, is a potential therapeutic target in ovarian carcinoma. *Proc. Natl Acad. Sci. USA* **112**, 232–237 (2015).
136. Lord, C. J. & Ashworth, A. The DNA damage response and cancer therapy. *Nature* **481**, 287–294 (2012).
137. Muller, F. L. et al. Passenger deletions generate therapeutic vulnerabilities in cancer. *Nature* **488**, 337–342 (2012).
This computational analysis of the The Cancer Genome Atlas glioblastoma dataset provides the first examples of the phenomenon of 'collateral damage', a passenger gene deletion that results in a novel dependency, and describes *ENO1*–*ENO2* in glioblastoma as a putative example.
138. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03435250 (2019).
139. Finn, R. S. et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. *Lancet Oncol.* **16**, 25–35 (2015).
140. Finn, R. S. et al. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. *Breast Cancer Res.* **11**, R77 (2009).
141. DeMichele, A. et al. CDK 4/6 inhibitor palbociclib (PD0332991) in Rb+ advanced breast cancer: phase II activity, safety, and predictive biomarker assessment. *Clin. Cancer Res.* **21**, 995–1001 (2015).
142. Turner, N. C. et al. Palbociclib in hormone-receptor-positive advanced breast cancer. *N. Engl. J. Med.* **373**, 209–219 (2015).
143. Finn, R. S., Aleshin, A. & Slamon, D. J. Targeting the cyclin-dependent kinases (CDK) 4/6 in estrogen receptor-positive breast cancers. *Breast Cancer Res.* **18**, 17 (2016).
144. Prahallad, A. et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* **483**, 100–103 (2012).
145. Kopetz, S. et al. Phase II pilot study of vemurafenib in patients with metastatic BRAF-mutated colorectal cancer. *J. Clin. Oncol.* **33**, 4032–4038 (2015).
146. Hyman, D. M. et al. Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. *N. Engl. J. Med.* **373**, 726–736 (2015).
147. Corcoran, R. B. et al. Combined BRAF, EGFR, and mek inhibition in patients with BRAF(V600E)-mutant colorectal cancer. *Cancer Discov.* **8**, 428–443 (2018).
148. Prahallad, A. et al. PTPN11 Is a central node in intrinsic and acquired resistance to targeted cancer drugs. *Cell Rep.* **12**, 1978–1985 (2015).
149. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03114319 (2019).
150. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03634982 (2019).
151. Whittaker, S. R. et al. Combined pan-RAF and MEK inhibition overcomes multiple resistance mechanisms to selective RAF inhibitors. *Mol. Cancer Ther.* **14**, 2700–2711 (2015).
152. Blumenschein, G. R. Jr et al. A randomized phase II study of the MEK1/MEK2 inhibitor trametinib (GSK1120212) compared with docetaxel in KRAS-mutant advanced non-small-cell lung cancer (NSCLC). *Ann. Oncol.* **26**, 894–901 (2015).
153. Janne, P. A. et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. *Lancet Oncol.* **14**, 38–47 (2013).
154. Hacohen, N., Fritsch, E. F., Carter, T. A., Lander, E. S. & Wu, C. J. Getting personal with neoantigen-based therapeutic cancer vaccines. *Cancer Immunol. Res.* **1**, 11–15 (2013).
155. Schumacher, T. N. & Schreiber, R. D. Neoantigens in cancer immunotherapy. *Science* **348**, 69–74 (2015).
156. Vinay, D. S. et al. Immune evasion in cancer: mechanistic basis and therapeutic strategies. *Semin. Cancer Biol.* **35**, S185–S198 (2015).
157. Wellenstein, M. D. & de Visser, K. E. Cancer-cell-intrinsic mechanisms shaping the tumor immune landscape. *Immunity* **48**, 399–416 (2018).
158. Zaretsky, J. M. et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N. Engl. J. Med.* **375**, 819–829 (2016).
This group discovered and described an acquired *JAK1* loss-of-function mutation in driving therapeutic resistance in a patient being treated with an anti-PD1 checkpoint inhibitor, the first example that tumour-intrinsic genetic mutations play a part in mediating immune cell killing.
159. Sade-Feldman, M. et al. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat. Commun.* **8**, 1136 (2017).
160. Ozcan, M., Janikovits, J., von Knebel Doeberitz, M. & Kloor, M. Complex pattern of immune evasion in MSI colorectal cancer. *Oncoimmunology* **7**, e1445453 (2018).
161. Grasso, C. S. et al. Genetic mechanisms of immune evasion in colorectal cancer. *Cancer Discov.* **8**, 730–749 (2018).
162. Dupuy, F. et al. LKB1 is a central regulator of tumor initiation and pro-growth metabolism in ErbB2-mediated breast cancer. *Cancer Metab.* **1**, 18 (2013).
163. Koyama, S. et al. STK11/LKB1 Deficiency promotes neutrophil recruitment and proinflammatory cytokine production to suppress T cell activity in the lung tumor microenvironment. *Cancer Res.* **76**, 999–1008 (2016).
This study is the first to associate loss of function of a known tumour suppressor gene (*LKB1*) with a suppressive immune phenotype in an engineered mouse tumour model. This finding was subsequently clinically validated in the retrospective analysis described in reference 164.
164. Skoulidis, F. et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. *Cancer Discov.* **8**, 822–835 (2018).
This analysis of a clinical study of patients with lung cancer treated with the anti-PD1 checkpoint inhibitor pembrolizumab is the first to provide clinical evidence that *LKB1* loss of function has a significant negative impact on the response to checkpoint inhibitors.
165. Layer, J. P. et al. Amplification of N-Myc is associated with a T cell-poor microenvironment in metastatic neuroblastoma restraining interferon pathway activity and chemokine expression. *Oncoimmunology* **6**, e1320626 (2017).
166. Kearney, C. J. et al. Tumor immune evasion arises through loss of TNF sensitivity. *Sci. Immunol.* **3**, eaar3451 (2018).
167. Peng, W. et al. Loss of PTEN promotes resistance to T cell-mediated immunotherapy. *Cancer Discov.* **6**, 202–216 (2016).
168. Hassel, J. C. et al. Combined immune checkpoint blockade (anti-PD-1/anti-CTLA-4): evaluation and management of adverse drug reactions. *Cancer Treat Rev.* **57**, 36–49 (2017).
169. Manguso, R. T. et al. In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. *Nature* **547**, 413–418 (2017).
This study demonstrates the utility of in vivo CRISPR genomic screening in a syngeneic mouse model to identify tumour-intrinsic critical nodes of immune modulation in solid tumours, and describes several specific genes that could be therapeutic targets designed to reverse the immune evasion phenotype.
170. Gao, J. et al. Loss of IFN-gamma pathway genes in tumor cells as a mechanism of resistance to anti-CTLA-4 therapy. *Cell* **167**, 397–404 e399 (2016).
171. Garcia-Diaz, A. et al. Interferon receptor signaling pathways regulating PD-L1 and PD-L2 expression. *Cell Rep.* **19**, 1189–1201 (2017).
172. Gilbert, L. A. et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell* **154**, 442–451 (2013).
173. Gilbert, L. A. et al. Genome-scale CRISPR-mediated control of gene repression and activation. *Cell* **159**, 647–661 (2014).
These authors discover and describe the utility of CRISPR-based gene silencing as an alternative to gene editing ('cutting') in genome-wide functional screens.
174. Qi, L. S. et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell* **152**, 1173–1183 (2013).
175. Zetsche, B. et al. Multiplex gene editing by CRISPR-Cpf1 using a single crRNA array. *Nat. Biotechnol.* **35**, 31–34 (2017).
176. Horlbeck, M. A. et al. Mapping the genetic landscape of human cells. *Cell* **174**, 953–967 e922 (2018).
177. Du, D. et al. Genetic interaction mapping in mammalian cells using CRISPR interference. *Nat. Methods* **14**, 577–580 (2017).
178. Han, K. et al. Synergistic drug combinations for cancer identified in a CRISPR screen for pairwise genetic interactions. *Nat. Biotechnol.* **35**, 463–474 (2017).
179. Wong, A. S. et al. Multiplexed barcoded CRISPR-Cas9 screening enabled by CombiGEM. *Proc. Natl Acad. Sci. USA* **113**, 2544–2549 (2016).
This is the first study to explore the interdependency of two individual genes using a multiplexed combo-CRISPR system.
180. Najm, F. J. et al. Orthologous CRISPR-Cas9 enzymes for combinatorial genetic screens. *Nat. Biotechnol.* **36**, 179–189 (2018).
181. Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A. & Liu, D. R. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* **533**, 420–424 (2016).
182. Gao, X. et al. Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents. *Nature* **553**, 217–221 (2018).
183. Nishida, K. et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. *Science* **353**, aaf8729 (2016).
184. Gaudelli, N. M. et al. Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. *Nature* **551**, 464–471 (2017).
185. Abudayyeh, O. O. et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science* **353**, aaf5573 (2016).
186. Abudayyeh, O. O. et al. RNA targeting with CRISPR-Cas13. *Nature* **550**, 280–284 (2017).
187. Cox, D. B. T. et al. RNA editing with CRISPR-Cas13. *Science* **358**, 1019–1027 (2017).
188. Konermann, S. et al. Transcriptome engineering with RNA-targeting type VI-D CRISPR effectors. *Cell* **173**, 665–676 e614 (2018).
189. Yan, W. X. et al. Cas13d is a compact RNA-targeting type VI CRISPR effector positively modulated by a WYL-domain-containing accessory protein. *Mol. Cell* **70**, 327–339.e5 (2018).
190. Gootenberg, J. S. et al. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science* **360**, 439–444 (2018).
191. Myhrvold, C. et al. Field-deployable viral diagnostics using CRISPR-Cas13. *Science* **360**, 444–448 (2018).
192. Dixit, A. et al. Perturb-Seq: dissecting molecular circuits with scalable single-cell RNA profiling of pooled genetic screens. *Cell* **167**, 1853–1866.e17 (2016).
This article describes Perturb-Seq, a method of using single-cell transcriptional output as the readout for CRISPR-based functional genomic screens.

193. Adamson, B. et al. A multiplexed single-cell CRISPR screening platform enables systematic dissection of the unfolded protein response. *Cell* **167**, 1867–1882.e21 (2016).
194. Jaitin, D. A. et al. Dissecting immune circuits by linking CRISPR-pooled screens with single-cell RNA-Seq. *Cell* **167**, 1883–1896.e15 (2016).
195. Xie, S., Duan, J., Li, B., Zhou, P. & Hon, G. C. Multiplexed engineering and analysis of combinatorial enhancer activity in single cells. *Mol. Cell* **66**, 285–299.e5 (2017).
196. Datlinger, P. et al. Pooled CRISPR screening with single-cell transcriptome readout. *Nat. Methods* **14**, 297–301 (2017).
197. Mirza, M. R. et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. *N. Engl. J. Med.* **375**, 2154–2164 (2016).
198. Li, W. et al. MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. *Genome Biol.* **15**, 554 (2014).

Acknowledgements
The authors thank C.P. Johnson and K. Briggs for helpful discussions and preparation of the manuscript and F. Li and T. Teng for curation and analysis of the Project Achilles dataset. All are full-time employees of and shareholders in Tango Therapeutics.

Author contributions
B.W. and A.H. researched data for article and wrote the article. A.A. contributed to writing the article. All authors contributed substantially to discussion of the content and reviewed and edited the manuscript before submission:

Competing interests
A.H. and B.W. are employees of and shareholders in Tango Therapeutics. L.A.G. is an employee of and shareholder in Eli Lilly and Company and is a shareholder in Tango Therapeutics. A.A. is a shareholder in Tango Therapeutics, a consultant for AtlasMDX, Third Rock Ventures, Pfizer, ProLynx and Bluestar and a Genentech scientific advisory board member and receives grant support from Sun Pharma and AstraZeneca. Patents on the use of PARP inhibitors held jointly with AstraZeneca that A.A. has benefited from financially (and may do so in the future) through the ICR Rewards to Inventors Scheme include WO2014013231 (A1) — 2014-01-23, US2012135983 (A1) — 2012-05-31, US2012010204 (A1) — 2012-01-12, US2006142231 (A1) — 2006-06-29, WO2008020180 (A2) — 2008-02-21.

RELATED LINKS
Cancer Cell Line Encyclopedia: https://portals.broadinstitute.org/ccle
Project Achilles: https://depmap.org/portal/achilles/
Project DRIVE: https://oncologynibr.shinyapps.io/drive/
Project Score: https://score.depmap.sanger.ac.uk/