Harnessing synthetic lethal interactions in anticancer drug discovery

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Abstract | Unique features of tumours that can be exploited by targeted therapies are a key focus of current cancer research. One such approach is known as synthetic lethality screening, which involves searching for genetic interactions of two mutations whereby the presence of either mutation alone has no effect on cell viability but the combination of the two mutations results in cell death. The presence of one of these mutations in cancer cells but not in normal cells can therefore create opportunities to selectively kill cancer cells by mimicking the effect of the second genetic mutation with targeted therapy. Here, we summarize strategies that can be used to identify synthetic lethal interactions for anticancer drug discovery, describe examples of such interactions that are currently being investigated in preclinical and clinical studies of targeted anticancer therapies, and discuss the challenges of realizing the full potential of such therapies.

Standard chemotherapies for cancer were initially discovered on the basis of their ability to kill rapidly dividing cells, and thus some of their common side effects — such as hair loss, nausea and immunosuppression — are due to the toxicity to rapidly dividing normal tissues[1,2]. With the aim of identifying therapies that have greater effectiveness and fewer side effects, cancer research in the past two decades has largely focused on discovering tumour-specific traits that might be exploited for selective targeting.

Many of the resultant targeted anticancer agents that have been discovered and investigated in this time affect cell signalling molecules — such as receptor tyrosine kinases — that have a key role in tumour growth and survival[3,4]. The development of such therapies is one of the most active areas of drug development, but so far only a few have demonstrated clinical efficacy and received regulatory approval. Nevertheless, these targeted therapies have increased the survival of patients with previously intractable cancers — including chronic myelogenous leukaemia[5–9], non-small cell lung cancer[10–13], pancreatic cancer[14,15], renal cell carcinomas (RCCs)[16–25] and liver cancers[26,27] — either as first-line therapies or in patients who have relapsed after standard chemotherapy. However, limitations of the first generation of targeted therapies — including the development of resistance, and on- and off-target toxicities — have become apparent[28,29].

One anticancer drug discovery strategy that shows great promise in specifically targeting cancer cells that possess genetic mutations that are not present in normal cells is the exploitation of synthetic lethality[30–33]. This form of cell killing (also known as conditional genetics) takes its name from classical genetic studies in model organisms such as yeast[34], and is based on the interaction of two genes that both contribute, often nonlinearly, to an essential process or processes[35,36]. When either gene is mutated alone, the cell is viable (FIG. 1a); however, the combination of mutations in both of these genes results in lethality (FIG. 1b). This process is referred to as synthetic lethality because cells with both gene mutations are not viable, and so it is not possible to directly isolate such cells. Nevertheless, various approaches can be used to evaluate and target potential synthetic lethal interactions, as discussed in the next section.

The interactions revealed by synthetic lethality studies can indicate a range of both predicted and unexpected connections. In the most conceptually straightforward scenario, two parallel pathways both contribute to an essential process. Consequently, disruption of a gene in one pathway is non-lethal, as the alternative pathway can sufficiently maintain the essential process, whereas disruption of both pathways is lethal to the cell. In cases in which substantial knowledge of a particular process exists, as in DNA damage repair, some of these synthetic lethal interactions can be predicted without the need for extensive screening[37,38]. Synthetic lethality, however, need not result from obvious parallel pathways, with some interactions arising from gene products within the same pathway or within the same protein complex.

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doi:10.1038/nrd3374

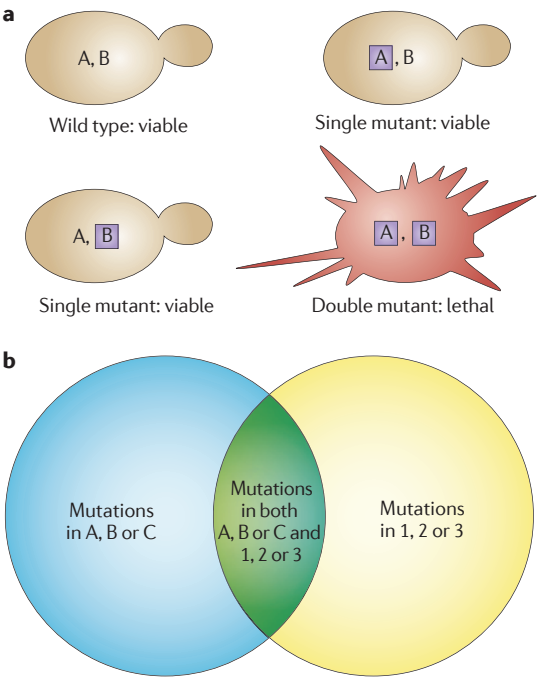


Figure 1 | **Synthetic lethality. a** | Organismal view. In model organisms, synthetic lethality describes the genetic interaction between two genes. If either gene is mutated by itself, the organism remains viable. The combination of a mutation in both genes is incompatible with viability and results in lethality. **b** | Pathway view. Two genes are considered to be synthetic lethal when they contribute to an essential process. For example, when either gene 'A', 'B' or 'C', or gene '1', '2' or '3' is mutated, the organism or cell remains viable. However, the combination of these mutations ('A', 'B' or 'C' with '1', '2' or '3') results in death.

Other associations may include two divergent pathways that are both needed for a response to a cellular insult, or a pathway that is only connected to another pathway as a result of a gain-of-function oncogenic mutation. High-throughput screening may prove to be particularly useful in identifying these more complex, unpredictable interactions.

Synthetic lethal targeting of cancer cells could be therapeutically advantageous to targeting of cancer cells with standard agents, in that only the cancer cells with a specific genetic mutation are killed; that is, it uses a genotype-selective toxin rather than a nonspecific cytotoxin. Cells without the cancer-inducing genotype are unaffected by such targeting, as inhibition of the targeted gene product does not affect cell viability and only the combination of an endogenous gene mutation in the cancer cell and targeted gene inhibition with a small interfering RNA (siRNA) or a small molecule leads to cell death. Exploiting synthetic lethality therefore increases selectivity towards killing tumour cells and, consequently, it enhances the therapeutic index between the tumour and normal tissue.

Beyond the potential for an enhanced therapeutic index, synthetic lethality offers the opportunity to exploit targets that have proved to be challenging to therapeutically modulate by other strategies. Although it is common to attempt to disrupt gain-of-function mutations in oncogenes[39–41], tumour growth in many cases is also driven by loss-of-function mutations in tumour suppressor genes. However, restoration of the function of tumour suppressor genes is not simple, as exemplified by the many attempts to restore their function by gene therapy[42–44]. The identification of synthetic lethal drugs and pathways could enable the exploitation of these gene mutations for selective targeting. In addition, not every oncogene proves to be directly tractable for pharmacological intervention. Here again, synthetic lethality may offer solutions by providing additional targets that take advantage of these driving mutations to provide a clinical benefit.

The utility of a synthetic lethality approach is further enhanced for several reasons. Specifically targeted agents based on synthetic lethality can be used as a form of monotherapy[45], in combination with cytotoxic chemotherapy and/or radiotherapy, or in patients with relapsed disease[46,47]. Furthermore, in some cases, the effect of a synthetic lethal interaction will be enhanced when combined with a genotoxic stress. Agents that induce synthetic lethality could theoretically accentuate the efficacy of cytotoxic therapies at lower doses, thereby decreasing off-target side effects and, consequently, increasing the therapeutic indices of either chemotherapies or radiation treatment. Moreover, as tumour progression is a multi-step process and the driving mutations change in the different stages of tumour growth, synthetic lethality could target a range of temporal mutations that occur from tumorigenesis to metastatic dissemination. Perhaps the greatest benefit of synthetic lethality is its potential to treat metastatic diseases, for which there are currently few effective and selective options.

In this Review, we first consider the advantages and disadvantages of different types of synthetic lethality screens for the discovery of potential anticancer drugs. We then discuss several case studies that exemplify progress in the discovery and development of such agents, including poly(ADP-ribose) polymerase (PARP) inhibitors in patients with *BRCA1* and/or *BRCA2* mutations, and compounds that could be effective in patients with mutations in *VHL*, *PTEN* and *RAS*.

Screening for mammalian synthetic lethality

Hartwell, Friend and colleagues[30] were the first to propose the concept of using synthetic lethality screening to identify new anticancer drugs. Recognizing that one of the limiting steps impeding drug discovery was the identification of tumour-selective characteristics, they suggested that loss-of-function mutations — such as those found in DNA repair genes or tumour suppressor genes — could be exploited. In the ideal setting, the first mutation would be a cancer-driving defect and highly conserved evolutionarily from model organisms to humans. Thus, by screening in yeast, synthetic lethal interactions could be identified, either by candidate selection or by genome-wide screening. Choosing putative targets requires both prior knowledge of involved pathways and the ability to generate specific mutations

Small interfering RNA
(siRNA). A sequence of double-stranded RNA, generally 21 nucleotides in length, which targets specific mRNA sequences for degradation or inhibits translation of specific genes. Synthetic siRNAs can be introduced into a cell by transfection but they are short-lived.

Poly(ADP-ribose) polymerase
(PARP). A family of enzymes that catalyses the conversion of nicotinamide adenine dinucleotide into nicotinamide and polymers of ADP-ribose at glutamic acid residues of nuclear proteins. These enzymes are involved in a variety of cellular processes, notably DNA repair.

to investigate the effects of various combinations of mutations on these pathways. By contrast, genome-wide screening is a blind, unbiased search that requires large-scale genetic screening technology.

As proof-of-concept, Hartwell *et al.*[30] performed a small-scale screen of a panel of 70 different isogenic strains from budding yeast with deletions in DNA damage response genes against US Food and Drug Administration (FDA)-approved chemotherapies. For validation of the feasibility of such a screen, the focus was on genetic instability as a basis for drug discovery. The rationale was that genetic instability is a common feature of many tumours, and that the genetic changes that underlie this genetic instability of tumour cells — in particular, defects in DNA damage response and repair pathways — could make tumour cells more sensitive to the effects of some drugs than normal cells. Hartwell, Friend and colleagues[30] were able to determine the drug sensitivities of two anticancer agents: cisplatin and mitoxantrone. Cisplatin demonstrated increased specificity for yeast strains that were defective in post-replication repair, whereas mitoxantrone — which functions as a topoisomerase II poison — resulted in increased sensitivity of yeast strains that were defective in double-stranded DNA break repair. This work demonstrated the feasibility of using large genetic screens to identify synthetic lethal interactions.

The value and applicability of synthetic lethality in the context of mammalian cells, especially in the cancer setting, is now more fully recognized. Efforts are no longer limited to model organisms nor are they limited to known essential cancer-driving pathways, such as those involved in genomic instability or DNA damage repair. Considerable technological advances have made it possible to screen for genes involved in synthetic lethal interactions in a mammalian setting (FIG. 2). Notably, the advent of libraries of either siRNAs or short hairpin RNAs (shRNAs), as well as combinatorial and diversity-oriented libraries of small molecules, enables genome-wide investigation of specific mutations in a rapid manner in mammalian cells.

There are fundamental similarities between screening either siRNA/shRNA libraries or small-molecule compounds to identify synthetic lethal interactions — both approaches frequently use matched, isogenic lines (in which an essential cancer gene has been identified) and a functional readout to assess whether an agent is cytotoxic, cytostatic or has no effect (FIG. 2). Both types of unbiased screens can also reveal unexpected connections that can directly and indirectly advance drug development efforts as well as basic research into our understanding of cancer biology. However, these two types of screens also have distinct although not necessarily mutually exclusive goals. The screening of RNA interference (RNAi)-based libraries can identify genes that are important in a pathway context and thus provide a better understanding of the fundamental biology behind interactions. By contrast, the goal of screening a small-molecule library is typically to obtain candidate compounds for the treatment of a given cancer genotype. Along with differing aims, there are advantages and disadvantages of

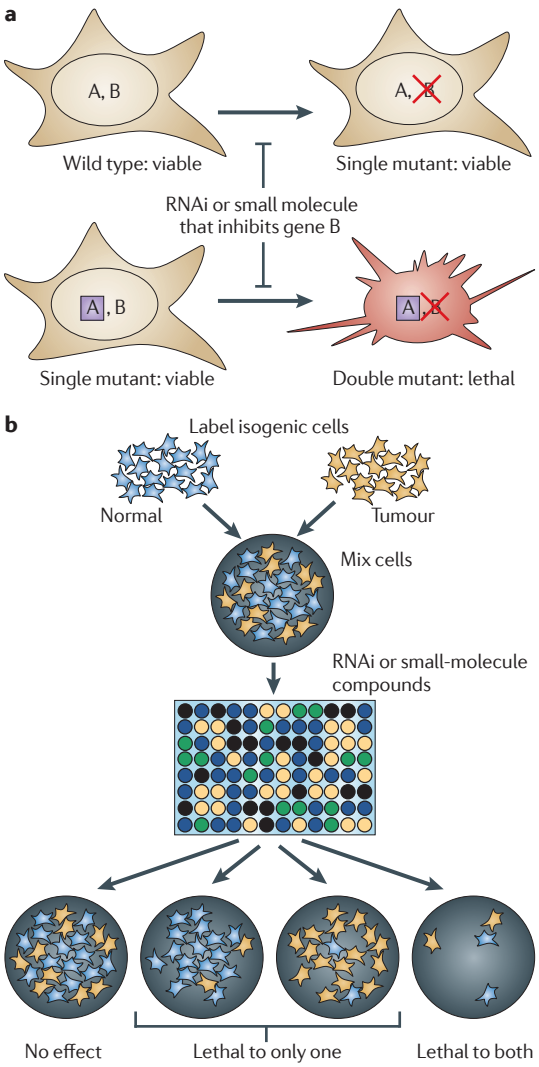


Figure 2 | **Mammalian synthetic lethality screens for anticancer efficacy. a** | Synthetic lethal screens can be used to identify genes or small-molecule compounds to specifically target tumour cells while sparing the normal tissue. A mutation in the first gene is essential to the development of cancer (for example, a loss-of-function mutation in a tumour suppressor gene or a gain-of-function mutation in an oncogene). The second gene would be identified either through an RNA interference (RNAi) library or it would directly be inhibited by a small-molecule compound. Inhibition of this second gene through RNAi or a small molecule alone would not interfere with tumour growth. Nonetheless, inhibiting the second gene in a tumour of a given genotype would result in selective cytotoxicity of the tumour. **b** | Isogenic cells that differ by only one essential cancer gene could be fluorescently tagged and mixed together in equal numbers. The cells would then be added to a 96-well plate and treated with a compound library. Fluorescence would be read over several days. Some compounds would not be toxic to either cell type; some would be selectively toxic to normal cells or selectively toxic to tumour cells; and some would be toxic to both genotypes. Validation of individual compounds through short-term and long-term survival assays — such as metabolic measurements or clonogenics — would then determine potential hits.

Short hairpin RNA
(shRNA). A plasmid or vector-based method for producing stable gene silencing. A promoter drives transcription of a target sequence, which forms a hairpin loop that is processed by the cellular RNA interference machinery, thereby forming small interfering RNAs to silence a particular gene.

high-throughput screening of either RNAi-based libraries or small-molecule compound libraries to identify synthetic lethal interactions (BOX 1).

RNAi-based screens allow for the direct discovery of unknown gene–gene interactions and pathways. However, the identification of such interactions and pathways does not necessarily lead readily to potential therapeutic candidates.

One approach for RNAi-based genome-wide screening requires a reverse transfection step, in which cells are plated onto pre-seeded plates containing the RNAi library along with a transfection reagent. Following incubation to allow for expression, the cells are then assayed for changes in viability. Off-target toxicity that is inherent to RNAi-based screening may be a source of false readouts. Alternative approaches for screening RNAi-based libraries include using plasmid vectors, in which cells are transfected with DNA encoding shRNA[48]. Viral methods, such as infection with retroviruses containing targeting sequences, can also be used for conditional inactivation of genes[49]. These plasmid-based approaches offer another advantage — the inclusion of a DNA 'bar code', which is a unique sequence for each shRNA-encoding plasmid that can be amplified from a mixed population. The abundance of each bar code within a pool under different conditions reveals the effect of an individual shRNA on survival and growth without the need to assay each plasmid individually.

Another important consideration is that the primary hits obtained from an RNAi library screen require a second screen to identify the agents that target the identified genes or their products[50]. If the synthetic lethal interactor is a known gene, then compounds to inhibit its activity may already exist[51]. Although knowing what the genetic target is for a synthetic lethal interaction could indicate the mechanism of action and aid in the development of small-molecule therapeutics, focusing on the known function of the target gene could also be misleading, as other mechanisms that are independent of the known function of the gene could be responsible for the synthetic lethality. Identifying a pathway involved in synthetic lethal interactions also provides additional targets for small-molecule inhibitors.

In contrast to RNAi libraries, identification of small molecules that demonstrate synthetic lethality in a screen directly provides potential candidates for optimization into lead compounds (for example, by improving potency or pharmacokinetic properties) without additional steps. In addition, the identification of the target of the small molecule can be valuable in structural modelling or for investigating structure–activity relationships to generate additional analogues with improved characteristics. However, this can require substantial additional effort compared to the RNAi-based screens for which the target is already known. A second screen using RNAi may be necessary to reveal the target (or targets).

It should be noted that using isogenic cell lines is not without challenges and is not the only approach that can be taken for synthetic lethality screens. Although this approach is technically and conceptually attractive for identifying synthetic lethal interactions with common, known cancer mutations, the single genetic variation being studied may not actually be the only difference in 'isogenic cells', which is a confounding factor in these types of studies. Rather, genetic drift between pairs of isogenic cell lines may result in multiple differences that can alter responses to RNAi or drug treatment. The problem of genetic drift may be especially acute when the mutation of interest results in a defect in DNA repair or genomic instability.

An alternative to isogenic lines has been established by Canaani and colleagues[52–54]; in this strategy, a single human cancer cell line that is deficient in a gene of interest is used. Complementation of this gene is provided by a low-copy unstable episome expressing this gene. In the context of a drug or RNAi screen, retention of this episome is selected under synthetic lethal conditions, thus revealing novel interactions. Although isogenic lines often provide a valuable avenue for synthetic lethality screens, this work demonstrates that other approaches also have distinct advantages.

Conditional synthetic lethality screens. To date, synthetic lethality screens have focused on specific, fixed genetic mutations and ignored transient yet unique features that can also be exploited. Conditional synthetic

Box 1 | **Advantages and disadvantages of RNAi libraries or small-molecule compound libraries**

There are certain considerations when screening for synthetic lethal interactions using RNA interference (RNAi) libraries or small-molecule compound libraries. Below, we list some advantages and disadvantages of these two approaches for the identification of synthetic lethal interactions.

RNAi libraries

- A top-down approach allows direct target identification
- An interaction may not necessarily lead to a therapeutic; that is, a compound to inhibit or activate the identified interaction target may not exist
- Nonspecific toxicity related to RNAi may lead to false negatives

Small-molecule compound libraries

- Directly provide potential candidates for optimization into lead compounds
- A bottom-up approach means that a target must be identified; therefore it does not immediately provide any new information on the biology of the disease or genetic interactions
- Amenable to structure–activity relationship analysis, which could help in optimizing compounds and identifying approaches to combat drug resistance

lethality screening demonstrates great potential by using interactions based on temporary situations to further increase the therapeutic index and the selectivity for cancer cells. Conditional synthetic lethality can develop in several different contexts — for example, in response to ionizing radiation, cytotoxic chemotherapeutic agents, or changes in the cellular microenvironment. Recent studies by Bindra *et al.*[55] and Chan *et al.*[56] have demonstrated that tumour hypoxia decreases the expression of homologous recombination proteins such as the DNA repair protein RAD51. Therefore, by suppressing the expression of DNA repair proteins, hypoxia conditionally transforms cells into a recombinational-deficient state and consequently they are sensitive to PARP inhibitors[57].

The impact of this conditional state of recombination deficiency induced by hypoxia should be common to most solid tumours, and could potentially be enhanced by agents that selectively increase tumour hypoxia by altering their metabolism. For example, treatment of transplanted tumours in mice with dichloroacetate (DCA) increases pyruvate consumption in the mitochondria and total oxygen consumption, which increases tumour hypoxia[58]. As DCA is already in clinical trials, it represents a practical approach for increasing tumour hypoxia, decreasing the expression of recombination proteins and sensitizing tumour cells to agents such as PARP inhibitors that will induce synthetic lethality[59]. This approach represents one example of how conditional synthetic lethal interactions can potentially be manipulated and exploited for cancer therapy (FIG. 3).

Case studies of synthetic lethality

In this section, selected case studies that exemplify the discovery and development of anticancer agents using synthetic lethality approaches are discussed.

DNA repair and synthetic lethality: PARP inhibitors and BRCA1/2 deficiency. Inhibitors of PARP are one of the first classes of small-molecule compounds that have been identified to interact in a synthetic lethal manner with mutations in the genes encoding proteins involved in DNA repair[60–64]. The tumour suppressor proteins BRCA1 and BRCA2 are important for the repair of double-stranded DNA breaks via homologous recombination, and mutations in their genes are associated with hereditary forms of breast and ovarian cancers[65–68]. PARP, however, functions as a sensor to recognize and recruit DNA repair proteins to the sites of single-stranded DNA breaks[69]. Given that unrepaired single-stranded DNA breaks result in stalled replication forks, and that one of the major roles of homologous recombination is to repair such stalled replication forks, tumours with impaired BRCA1 or BRCA2 function and, consequently, compromised homologous recombination function, could be sensitized to inhibitors of enzymes involved in single-stranded DNA break repair, such as PARP inhibitors[70].

Indeed, inhibition of PARP with such compounds was discovered to result in synthetic lethal interactions with mutations in *BRCA1* and *BRCA2* (REFS 37,38). Bryant *et al.*[37] and Farmer *et al.*[38] demonstrated that inhibition of PARP1 in conjunction with either BRCA1 or BRCA2 deficiency resulted in a failure to repair double-stranded DNA breaks and recombination lesions, leading to apoptosis. Cells that were deficient in BRCA1 or BRCA2 or cells in which BRCA1 or BRCA2 were depleted by shRNAs were sensitive to PARP inhibition, both pharmacologically and genetically. Furthermore, BRCA2-deficient xenografts were also sensitive to PARP inhibitors. The conditional genetic interaction between *BRCA* deficiency and PARP inhibition provides proof-of-principle that synthetic lethal relationships can be identified for targeting homologous recombination and other DNA repair pathways (BOX 2).

The combination of PARP inhibition and loss of BRCA1 or BRCA2 function represents an exciting approach to specifically exploit DNA repair deficiencies in tumour cells. Based on these findings, PARP inhibitors are now being evaluated in clinical trials in patients with breast and ovarian cancers that are characterized by *BRCA1* or *BRCA2* mutations[46,71,72]. In addition to breast and ovarian cancers, PARP inhibitors are also being developed by a number of pharmaceutical companies and are being tested clinically in a variety of tumour types (TABLES 1,2).

As well as potentially having potent activity as single agents in patients with defective BRCA1 or BRCA2 function[73], sensitizing tumour cells by targeting DNA repair deficiencies with PARP inhibitors could also allow the use of lower doses of existing cytotoxic drugs or ionizing radiation and, consequently, reduced side effects[74,75]. For example, standard DNA-damaging drugs for which the resultant DNA lesions are repaired by recombination repair, such as mitomycin C or cisplatin, are ideal candidates for combination therapy with PARP inhibitors in BRCA1 or BRCA2 recombination-deficient cells[76–81]. Several clinical trials are examining the efficacy of PARP inhibitors with various DNA-damaging agents (TABLES 1,2). Interestingly, Fong *et al.*[82] demonstrated that PARP inhibitors were effective in patients with *BRCA1*- and *BRCA2*-mutant ovarian cancers. More importantly, response to PARP inhibition was directly correlated to sensitivity to platinum-based therapies[82,83]. These findings demonstrate that synthetic lethal interactions can be used to predict clinical efficacy and thus determine which patients are more likely to have a durable response to a particular treatment.

DNA repair and synthetic lethality: MSH and DNA polymerases. Although *BRCA1* and *BRCA2* mutations and their impact on homologous recombination have been well documented in past studies, further studies should be carried out to explore the possibility that tumour cells may have other defects in DNA repair pathways, which could be exploited in a similar manner[84,85]. For example, although DNA repair pathways are impaired in BRCA2-deficient cells, Feng *et al.*[86] determined that RAD52 mediated an alternative homologous recombinational repair pathway. Depletion of RAD52 substantially reduced homologous recombination and increased cell death independently of BRCA2. However, the combination of RAD52 and BRCA2 deficiencies

Replication fork
The structure formed from the unwinding and breaking of the hydrogen bond of the two strands of DNA during replication. Each individual strand of DNA becomes a template for replication.

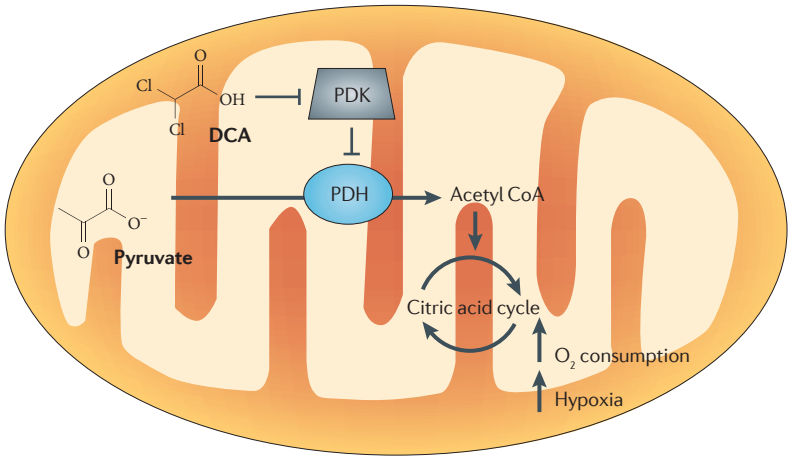


Figure 3 | **Example of a conditional synthetic lethality opportunity.** Dichloroacetate (DCA) inhibits the function of pyruvate dehydrogenase kinase (PDK) in mitochondria, relieving the inhibition of pyruvate dehydrogenase (PDH). PDH catalyses the conversion of pyruvate to acetyl CoA, which is an important substrate of the citric acid cycle. When PDH is active, the citric acid cycle utilizes oxygen, increasing oxygen consumption and consequently resulting in hypoxia. The hypoxic condition can then be targeted by synthetic lethality.

resulted in a synthetically lethal interaction, demonstrating that targeting alternative homologous recombination pathways is an effective strategy for targeting tumour cells.

To screen for patients who have repair defects and would benefit from this form of targeted therapy, an assay could be used to detect decreased DNA repair protein foci formation in biopsy specimens that have been irradiated *ex vivo*[87]. For example, immunofluorescence of the DNA repair proteins BRCA1, FANCD2 or RAD51 (REF. 87) would allow for the detection of tumours with decreased expression of DNA repair proteins that may not necessarily be due to mutations in DNA repair genes, which is a rare occurrence in tumours. Defective or decreased DNA recombination activity should be equally sufficient for synthetic lethal targeting as a therapeutic strategy.

Recently, Martin *et al.*[88,89] identified a pair of potential synthetic lethal interactions with DNA mismatch repair proteins. In a cell line and in several patient samples, MSH2-deficiency correlated with an increase in DNA polymerase β (Pol β), whereas deficiency in MLH1 correlated with an increase in Pol γ[88]. *MSH2* and *MLH1* are two genes that encode DNA mismatch repair proteins and are associated with hereditary nonpolyposis colorectal carcinomas through their DNA mismatch repair activities[90–94]. Pol β[95–97] and Pol γ[98,99] are two DNA polymerases that are involved in base excision repair. Silencing of either Pol β or Pol γ in an *MSH2*-negative or *MLH1*-negative cell line increased nuclear or mitochondrial 8-oxoguanine (8-oxoG) levels, respectively, which indicates a failure to repair oxidized nucleotides.

In a separate report, Martin *et al.*[89] also determined that depletion of PTEN-induced putative kinase 1 (PINK1) and other mitochondrial kinases increased the production of reactive oxygen species, as well as formation of 8-oxoG. Accumulation of the oxidized base, 8-oxoG, causes DNA base lesions through incorporation of either cytosine or adenine bases, which can cause GC→TA transversions. Base excision repair is one of the primary methods of dealing with 8-oxoG lesions. Thus, simultaneous inhibition of mismatch repair and DNA polymerase proteins leads to an accumulation of DNA damage by interfering with base excision repair, which results in cell death.

As MLH1 and MSH2 are defective in a number of nonhereditary colorectal cancers, Martin and colleagues propose that Pol β inhibitors could be used for a defined period of time rather than for the chronic treatment of colon carcinoma to limit the development of drug resistance. Furthermore, distinct treatments with a given end point would also prevent the accumulation of additional mutations through inhibition of DNA repair pathways. This study[89] highlights that although synthetic lethal interactions may be readily identified, target identification plays only a small part in developing targeted therapies.

VHL. *VHL* is a tumour suppressor gene that follows the Knudson two-hit hypothesis and is associated with a tumour-prone syndrome that is characterized by a unique distribution of tumours, including haemangioblastomas of the central nervous system, retinal angiomas, pancreatic islet cell tumours, phaeochromocytomas of the adrenal glands and renal tumours[100,101]. In addition, it is estimated that *VHL* mutation or gene silencing via methylation accounts for over 80% of spontaneous and hereditary kidney tumours[102–104]. Renal tumours are particularly intractable as they are difficult to diagnose because they do not generally present with clinical symptoms until after they have metastasized to distant sites[105].

Knudson two-hit hypothesis
A model that proposes that cancer is a genetic disease and that successive genetic alterations in both alleles are needed to turn a normal cell into a cancer cell. In spontaneous cancers, two successive rare events must occur but in cases of hereditary susceptibility to cancer, inheritance of a damaged gene followed by a rare event results in mutation.

Box 2 | **DNA repair pathways**

Poly(ADP-ribose) polymerase (PARP) inhibitors are the most clinically developed synthetic lethal targeting agents to date for killing cells with *BRCA* mutations. BRCA is normally involved in homologous recombinational repair of double-stranded DNA breaks, whereas PARP has a role in single-stranded DNA damage repair (base excision repair). Impairment of a combination of single- and double-stranded DNA break repair should result in synthetic lethality.

Single-stranded DNA damage

- Base excision repair: fixes single base damage caused by oxidation, alkylation, hydrolysis or deamination
- Nucelotide excision repair: fixes bulky, helix-distorting lesions including pyrimidine dimers; includes transcription-coupled repair in which genes that are actively being transcribed are fixed
- Mismatch repair: fixes mispaired nucleotide errors during replication or recombination

Double-stranded DNA breaks

- Non-homologous end joining: end breaks are directly ligated
- Homologous recombination: identical or nearly identical sequences are used as a template to fix the break

Table 1 | **Clinical trials of PARP inhibitors***

Disease site	Disease type	Additional agents	Phase (ClinicalTrials.gov identifier)
Iniparib/BSI-201/SAR240550 (BiPar Sciences/Sanofi-Aventis)			
Breast	Triple negative (local and metastatic)	Carboplatin (Paraplatin; Briston-Myers Squibb) and gemcitabine (Gemzar; Eli Lilly & Co.)	II/III (NCT01045304; NCT00540358; NCT00813956; NCT00938652)
Breast	Triple negative (local and metastatic)	Irinotecan (Camptosar; Pfizer)	II (NCT01173497)
Breast	Triple negative (local and metastatic)	Paclitaxel	II (NCT01204125)
Breast	Triple negative (local and metastatic)	None	II (NCT01130259)
Glioblastoma	Not specified	Temozolomide (Temodar; Merck)	I/II (NCT00687765)
Lung	Non-small cell (stage IV)	Cisplatin and gemcitabine	II (NCT01086254)
Lung	Squamous cell	Carboplatin and gemcitabine	III (NCT01082549)
Ovarian	*BRCA1* and/or *BRCA2* mutation	None	II (NCT00677079)
Ovarian	Recurrent, platinum-resistant or platinum-sensitive	Carboplatin and gemcitabine	II (NCT01033292; NCT01033123)
Uterine	Not specified	Carboplatin and paclitaxel	II (NCT00687687)
CEP-9722 (Cephalon)			
Solid tumours	Not specified	Temozolomide	I (NCT00920595)
MK-4827 (Merck)			
Solid tumours	Glioblastoma, melanoma	Temozolomide	I (NCT01294735)
Solid tumours	Not specified	Carboplatin, paclitaxel and liposomal doxorubicin	I (NCT01110603)
Solid tumours	Ovarian or prostate cancer	None	I (NCT00749502)
Solid tumours	Ovarian cancer	Pegylated liposomal doxorubicin	I (NCT01227941)
PF-01367338/AG014699 (Pfizer)			
Breast	*BRCA* mutation; triple negative (local or metastatic)	Cisplatin	II (NCT01074970)
Breast, ovarian	*BRCA* mutation	None	II (NCT00664781)
Solid tumours	Not specified	Carboplatin, paclitaxel, cisplatin, pemetrexed (Alimta; Eli Lilly & Co.), epirubicin (Pharmorubicin; Pfizer) and cyclophosphamide	I (NCT01009190)
Olaparib/KU-0059436/AZD2281 (KuDOS/AstraZeneca)			
Breast	*BRCA* mutation	None	II (NCT00494234)
Breast, ovarian	Triple negative (metastatic)	Carboplatin and paclitaxel	I (NCT00516724; NCT00707707; NCT00516724)
Breast	*BRCA* mutation; sporadic, triple negative	Carboplatin	I (NCT00647062)
Colon	Not specified	Irinotecan	I (NCT00535353)
Colon	Not specified	None	II (NCT00912743)
Stomach	Not specified	Paclitaxel	II (NCT01063517)
Ovarian	*BRCA* mutation	None	I/II (NCT00516373; NCT00494442)
Ovarian	*BRCA* mutation	Liposomal doxorubicin	II (NCT00628251)
Breast, ovarian	Platinum-sensitive	Carboplatin and paclitaxel	I/II (NCT00516724; NCT00647062)
Pancreas	Not specified	Gemcitabine	I (NCT00515866)
Solid tumours	Not specified	Cisplatin	I (NCT00782574)

*Data are taken from ClinicalTrials.gov. Poly(ADP-ribose) polymerase (PARP) inhibitors are the most clinically developed synthetic lethal targeting agents, killing cells with *BRCA* mutations. BRCA normally functions in homologous recombinational repair of double-stranded DNA breaks, and PARP plays a part in single-stranded DNA damage repair (base excision repair). Impairment of a combination of single- and double-stranded DNA break repair should result in synthetic lethality.

Table 2 | **Clinical trials of PARP inhibitors*: veliparib/ABT-888 (Abbott)**

Disease site	Disease type	Additional agents	Phase (ClinicalTrials.gov identifier)
Brain	Not specified	Whole brain radiation	I (NCT00649207)
Brain	Not specified	Temozolomide (Temodar; Merck)	I (NCT00946335)
Brain	Not specified	Temozolomide	I/II (NCT00770471; NCT01026493)
Breast	Metastatic, *BRCA1* and/or *BRCA2* mutation	Temozolomide	II (NCT01009788)
Breast	HER2 (also known as ERBB2)-negative	Carboplatin (Paraplatin; Briston-Myers Squibb)	I (NCT01251874)
Breast	*BRCA1* and/or *BRCA2* mutation (stage III/IV)	Carboplatin	II (NCT01149083)
Breast	*BRCA1* and/or *BRCA2* mutation	Gemcitabine (Gemzar; Eli Lilly & Co.)	I (NCT01154426)
Breast	ER-negative, PR-negative, triple negative, metastatic (stage III/IV)	Cisplatin and vinorelbine (Navelbine; Pierre Fabre SA)	I (NCT01104259)
Breast	Not specified	Carboplatin, paclitaxel	I (NCT01281150)
Breast	Not specified	Neratinib (HKI-272; Pfizer)	II (NCT01042379)
Cervix	Not specified	Filgrastim (Neupogen; Amgen), pegrastim and topotecan (Hycamptin; GlaxoSmithKline)	II (NCT01266447)
Colon	Male, non-resectable	Temozolomide	II (NCT01051596)
Colon	Male, non-resectable	5-fluorouracil, irinotecan (Camptosar; Pfizer) and levofolinic acid	I (NCT01123876)
Colon	Not specified	5- fluorouracil, irinotecan and levofolinic acid	I (NCT01123876)
Leukaemia	Not specified	Temozolomide	I (NCT01139970)
Leukaemia, lymphoma, ovarian and breast	*BRCA*-positive, triple negative; non-Hodgkin's lymphoma	Metronomic cyclophosphamide	II (NCT01306032)
Liver	Not specified	Temozolamide	II (NCT01205828)
Lymphoma	Not specified	Carboplatin and topotecan	I (NCT00588991)
Lymphoma	Not specified	Irinotecan	I (NCT00576654)
Ovarian	After platinum-based first-line chemotherapy	Topotecan	I/II (NCT01012817)
Ovarian	After platinum-based first-line chemotherapy	Bevacizumab (Avastin; Roche/Genentech), carboplatin and paclitaxel	I (NCT00989651)
Prostate	Not specified	Temozolamide	I (NCT01085422)
Solid tumours	Metastatic melanoma, breast, ovarian, fallopian or hepatocellular cancer	Temozolomide	I (NCT00526617)
Solid tumours	Prostate cancer	Temozolomide	I (NCT01085422)
Solid tumours	Liver cancer	Temozolomide	II (NCT01205828)
Solid tumours	Ovarian cancer	Temozolomide and pegylated liposomal doxorubicin	II (NCT01113957)
Solid tumours	Not specified	Temozolomide	II (NCT01193140)
Solid tumours	*BRCA1* and/or *BRCA2* mutation; breast, fallopian, ovarian, pancreatic or prostate cancer	None	I (NCT00892736)
Solid tumours	Not specified	Mitomycin C	I (NCT01017640)
Solid tumours	Breast, ovarian or fallopian cancer	Pegylated liposomal doxorubicin	I (NCT01145430)
Solid tumours	*BRCA1* and/or *BRCA2* mutation; ovarian cancer	Carboplatin and paclitaxel	I (NCT00535119)
Solid tumours	Not specified	Carboplatin and gemcitabine	I (NCT01063816)
Solid tumours	*BRCA1* and/or *BRCA2* mutation; breast, colorectal, gastric, ovarian or pancreatic cancer	Capecitabine (Xeloda; Roche) and oxaliplatin (Eloxatin; Sanofi-Aventis)	I (NCT01233505)
Solid tumours	Bladder, gall bladder, extrahepatic bile duct, liver, lung, pancreatic or transitional cell cancer of renal pelvis and ureter	Cisplatin and gemcitabine	I (NCT01282333)

ER, oestrogen receptor; PR, progesterone receptor. *Data are taken from ClinicalTrials.gov. Poly(ADP-ribose) polymerase (PARP) inhibitors are the most clinically developed synthetic lethal targeting agents, killing cells with *BRCA* mutations. BRCA normally functions in homologous recombinational repair of double-stranded DNA breaks, and PARP plays a part in single-stranded DNA damage repair (base excision repair). Impairment of a combination of single- and double-stranded DNA break repair should result in synthetic lethality.

RCC has also been reported to be resistant to both radiation therapy and standard chemotherapeutic agents[106]. Owing to the strong relationship between loss of *VHL* function and the development of RCC[107], and the lack of curative treatments, renal cancer cell lines that contain *VHL* mutations are attractive tools for identifying agents that function in a synthetically lethal manner.

High-throughput screening of shRNA libraries and small-molecule compound libraries have both been used to uncover genetic interactions with mutant *VHL*[108,109]. Bommi-Reddy *et al.*[108] used 100 shRNA vectors directed against 88 different kinases to identify those that could inhibit proliferation of cells lacking VHL function. This study found three kinases that were capable of preferentially impairing growth of *VHL*-mutant cells in a dose-dependent manner: cyclin-dependent kinase 6 (CDK6), hepatocyte growth factor receptor (also known as MET) and dual specificity mitogen-activated protein kinase kinase (MAP2K1). Furthermore, pharmacological inhibition of CDK4 and/or CDK6 reduced the viability of *VHL*-mutant cells, providing proof-of-principle that screening of shRNA panels can detect synthetic lethal interactions in tumour cells.

In a separate study by Turcotte *et al.*[109], in which 64,000 compounds were screened using a fluorescent cell-based growth assay, one compound — STF-62247 — was found to be selectively cytotoxic to cells lacking VHL. This candidate was then confirmed and validated in more stringent assays, such as clonogenic survival curves. Treatment of *VHL*-mutant cells with STF-62247 induced autophagy[109]. The yeast genome deletion pool was used to identify the target of STF-62247; this revealed a previously unknown relationship between Golgi trafficking, autophagy and the loss of VHL function. This study emphasizes the advantages and disadvantages of a small-molecule screen[110,111].

From a clinical perspective, although there have recently been important advances in the treatment of RCC with drugs that target vascular endothelial growth factor signalling (which can be upregulated by loss of VHL function) and mammalian target of rapamycin (mTOR), this study also highlights a promising opportunity to improve the effectiveness of strategies to treat RCC by more directly exploiting the presence of the underlying VHL mutations. The recent identification of frequent mutations in the SWI/SNF chromatin remodelling complex gene *PBRM1* offers another opportunity for targeting RCC[112].

Targeting the loss of PTEN. In addition to VHL and BRCA, other loss-of-function tumour suppressor mutations have been examined for synthetic lethal interactions. Several studies have demonstrated a synthetic lethal interaction between mTOR inhibition and PTEN loss[113–115]. *PTEN* is a tumour suppressor gene that is frequently mutated in both hereditary and spontaneous cancers. Loss of PTEN increases activation of AKT, which in turn increases proliferation and protein synthesis, and decreases apoptosis. Treatment with CCI-779 (also known as temsirolimus (Torisel; Pfizer)), an mTOR inhibitor, was selectively toxic to PTEN-deficient cell lines as well as *PTEN*-heterozygous and *PTEN*-homozygous mouse tumours[113,114]. CCI-779, an analogue of rapamycin, is a particularly attractive therapeutic compound because it already has FDA approval for the treatment of RCC (see above). CCI-779 decreased cell proliferation and cell size, while increasing apoptosis by inhibiting activation of both AKT and ribosomal protein S6 kinase.

Thomas and colleagues[115] further demonstrated that growth arrest caused by CCI-779 correlated with a block in translation of hypoxia-inducible factor mRNA in VHL-deficient cells. Their results suggest that synthetic lethal interactions contribute to the clinical efficacy of some current drugs and, importantly, that an understanding of these interactions can guide their application to those patients who have mutations in their tumours that make them more responsive to these drugs than other patients.

These studies have focused on clinically approved compounds. However, large-scale synthetic lethal screening based on loss of PTEN is complicated by the fact that restoration or overexpression of PTEN to generate matched isogenic cell lines results in cell cycle arrest. Using PTEN-null cells in a cell-based assay of forkhead box protein O1A (FOXO1A) nuclear localization, Kau *et al.*[116] were able to circumvent this limitation by examining a downstream consequence of PTEN loss. In cells lacking PTEN, FOXO1A is inactivated owing to its mislocalization to the cytoplasm, but redirecting FOXO1A to the nucleus can reverse the promotion of tumour growth caused by loss of PTEN. Kau *et al.* therefore screened for inhibitors of FOXO1A nuclear export, and they found several classes of compounds, including general nuclear export inhibitors and antagonists of calmodulin signalling.

Shen *et al.*[117] recognized that one of the functions of PTEN is to maintain genomic stability by repairing double-stranded DNA breaks via homologous recombination. Thus, in addition to breast and ovarian cancers with *BRCA* mutations, a variety of tumour types, including endometrial cancers and glioblastomas, should also be considered in clinical studies with PARP inhibitors[118–120]. These studies on the loss of PTEN also illustrate how downstream functions of genes of interest can be used in the development of synthetic lethality strategies.

***Targeting dysregulation of* RAS *and* MYC.** Although the synthetically lethal compounds that have progressed furthest in the clinic so far target loss-of-function mutations in tumour suppressor genes, gain-of-function mutations in oncogenes such as *RAS* can also be targeted. It is estimated that a quarter of all cancers have activating *RAS* mutations, with half of some specific cancers, such as colorectal carcinoma, having gain-of-function *RAS* mutations[121]. A number of synthetic lethality screens with activated *RAS* have been undertaken to circumvent the pharmacological intractability of oncogenic *RAS*[122,123]. Torrance and colleagues[124] demonstrated a proof-of-principle synthetic lethality screen using isogenically matched cell lines with and without the mutant *KRAS* oncogene. Colon carcinoma cell lines containing

Clonogenic survival curve
The standard method for determining the effectiveness of a particular treatment on the proliferation of cells. Cells are plated in a tissue culture dish and allowed to attach overnight. The plates are then treated and grown until single cells form colonies, which are then fixed, stained and counted.

Autophagy
A catabolic process that sequesters and recycles cellular components, including organelles and long-lived proteins, in response to diverse stimuli. Autophagosomes form via invagination of the cell membrane, creating double-membrane vesicles. These autophagic vesicles then fuse with lysosomes, creating autophagolysosomes in which the contents of the cell are degraded by acidic lysosomal hydrolases.

the mutant *KRAS* were engineered to express yellow fluorescent protein, whereas the matched cell line in which the mutant *KRAS* was eliminated by homologous recombination was engineered to express a blue fluorescent protein. These two matched cell lines were co-cultured and screened against approximately 30,000 compounds to identify small molecules that were selectively cytotoxic to cells containing mutant *KRAS*; this resulted in the identification of four selective compounds. Two previously known RAS pathway inhibitors with unknown mechanisms of action — demethoxyviridin (a wortmannin analogue) and mithramycin — were revealed, thus indicating that this screening method was feasible. Two compounds — triphenyltetrazolium (TPT) and a sulphinylcytidine derivative (SCD) — were also identified as novel RAS pathway inhibitors in this screen.

The authors performed a structural homology search of the two libraries they screened and found several small molecules that were structurally similar to TPT but were not identified in the screen. These compounds did not inhibit the growth of cells with mutant *KRAS*. Moreover, a known p38 mitogen-activated protein kinase (MAPK) inhibitor — SB203580 — which is structurally similar to TPT, had no selectivity for mutant RAS cells and, likewise, TPT had no effect on p38 MAPK. SCD was further analysed, as it inhibited growth in several additional isogenic cell lines containing mutant *KRAS*. Animals bearing $KRAS^{G13D}$-mutant tumours showed a reduction in tumour growth when they were treated with SCD, thus demonstrating that a cytosine nucleoside analogue could potentially be used to treat tumours with mutant *RAS*. The identification of these compounds could provide the basis for synthesizing more specific compounds through the optimization and analysis of structure–activity relationships, although this could be hampered by the lack of knowledge on how these compounds might function, which would require further investigation to identify their mechanism of action.

In an independent screen, Sarthy *et al.*[125] used an siRNA library to identify genes with synthetic lethal interactions with *KRAS* in the same colon carcinoma cell line. This study found that inhibiting expression of baculoviral IAP repeat-containing protein 5 (BIRC5; also known as survivin) in cells with an activated *KRAS* oncogene resulted in lower survival rates compared with isogenic cells in which *KRAS* was inhibited by siRNA. However, although this illustrates the value of genetic screening in identifying the importance of a particular genetic interaction, genetic screening does not necessarily readily indicate a therapeutically viable option with which to progress directly, given that antisense oligonucleotides and siRNA-based treatments are currently limited by delivery methods.

Using a genetic mouse tumour model of non-small cell lung carcinoma, Puyol *et al.*[126] identified a synthetic lethal interaction between *KRAS* and *CDK4*. Ablation of CDK4 but not CDK2 or CDK6 reduced the number and grade of $KRAS^{G12V}$-induced lung tumours. Genetic elimination or pharmacological inhibition of CDK4 slowed tumour growth by inhibiting proliferation and inducing cell senescence. This study provides genetic evidence to explain why CDK inhibitors alone have limited efficacy[127] and suggests that tumours with specific *KRAS* mutations may benefit from specific CDK inhibition.

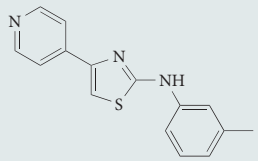
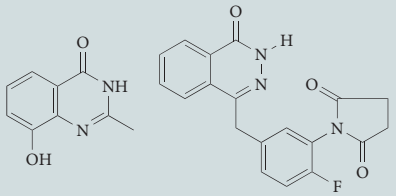
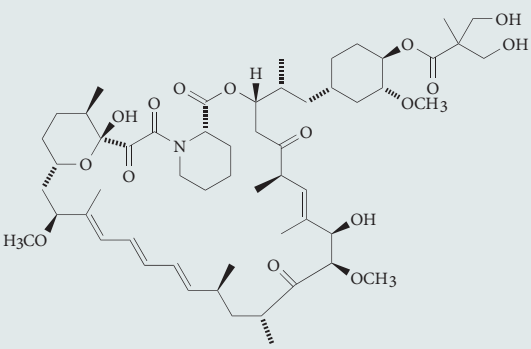
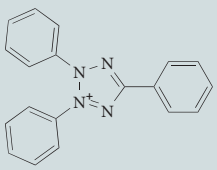
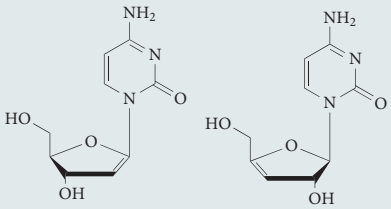
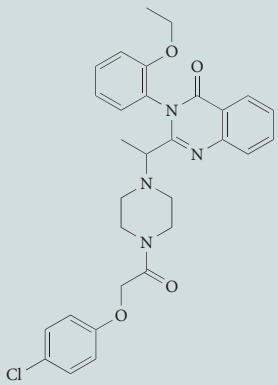
Oncogenic KRAS has also been shown to increase activation of the ataxia telangiectasia and RAD3-related protein (ATR)–CHK1 pathway and cause a subsequent increase in genomic instability[128]. Inhibition of ATR in the context of oncogenic RAS had two distinct outcomes depending on the degree of impairment. Haploinsufficiency of *ATR*, or a modest decrease, increased tumorigenesis. A further increase in ATR inhibition caused a decrease in cell viability and a shift to synthetic lethality, thus demonstrating the delicate balance that must be struck when targeting essential pathways.

More recent large-scale genomic screening of oncogenic RAS reveals the complexities in undertaking a synthetic lethality screen[122,123]. Using two different approaches, Luo *et al.*[51] and Scholl *et al.*[50] identified pathways that could be inhibited in *KRAS*-mutant cells, which resulted in decreased cell viability. Luo *et al.* used isogenic cells with either mutant or wild-type *RAS*, using a second pair of isogenic cells for the screening validation. Their initial screen did not identify an obvious 'hit'; rather, subsequent computational analysis indicated that the mitotic machinery or the proteasome had a synthetic lethal interaction with mutant *RAS*. The findings of this screen predicted and determined that cells with mutant *RAS* are sensitive to agents that target mitotic spindle function, such as the taxanes, or agents that inhibit the proteasome, such as bortezomib (Velcade; Millennium Pharmaceuticals/Centocor Ortho Biotech).

As this was an RNAi library screen, the potential for a cancer therapeutic relied on developed or known compounds. For example, the authors used a previously identified small-molecule inhibitor of polo-like kinase 1 (PLK1)[129], a mitotic kinase, to demonstrate that mitotic pertubation resulted in death of cells with mutant *RAS*. However, Scholl *et al.*[50] identified a single gene, *STK33* (REF. 130), for which there was no developed inhibitor. Rather, this RNAi screen identified a previously unknown interaction between *STK33* and mutated *KRAS*. Similarly, the genetic interplay between nuclear factor-κB (NF-κB) and oncogenic KRAS could potentially be targeted, as upstream regulators of NF-κB have been shown to be essential for KRAS expression[131] and transformation[132], whereas NF-κB signalling is essential for lung adenocarcinoma development[133].

In another study, Dolma *et al.* screened two chemical libraries for synthetic lethal interactions with oncogenic $HRAS^{V12}$ in engineered tumour cells derived from primary fibroblasts[134]. This screen identified a novel compound, erastin, which selectively killed tumorigenic cells expressing HRAS and simian virus 40 (SV40) small T oncoprotein through non-apoptotic cell death. In order to determine the mechanism of genotype-specific cytotoxicity, two subsequent approaches were taken: a suppressor screen with a battery of bioactive compounds of previously determined function and a functional screen based on affinity purification[135]. The suppressor

Table 3 | **Synthetic lethal interactions for the treatment of cancer**

Compound	Structure	Target	Genetic interaction	Proposed use	Refs
STF-62247		Autophagy	*VHL*	Renal cancer	109
NU1025 and KU0058684		Poly(ADP-ribose) polymerase	*BRCA1* and/or *BRCA2*	Breast cancer, ovarian cancer	37,38
CCI-779		Mammalian target of rapamycin	*PTEN*	Renal cancer	113–115
Triphenyltetrazolium		p38 mitogen-activated protein kinase	*RAS*	Colon cancer	124
Sulfinylcytidine derivatives		Cytosine nucleoside (replication and/or translation)	*RAS*	Colon cancer	124
Erastin		Voltage-dependent anion channels	*RAS*	Not stated	134,135
Death receptor 5 (DR5) agonist (monoclonal antibody)	Not applicable	DR5	*MYC*	Not stated	136

screen utilized 2,000 biologically active compounds with known targets and/or functions. Several antioxidants (for example, α-tocopherol, butylated hydroxytoluene and β-carotene) prevented erastin-induced death. Immobilization of an erastin analogue to a solid support followed by mass spectrometry revealed that erastin interacted with specific isoforms of voltage-dependent anion channels (VDACs). These two techniques showed that mitochondrial VDACs are responsible for an oxidative, iron-dependent, non-apoptotic cell death in response to erastin. More importantly, these studies identified a synthetic lethal interaction between VDAC and oncogenic $HRAS^{V12}$, the more common KRAS mutants and a promising lead compound.

Using a similar cell system of immortalized and transformed fibroblasts, Wang and colleagues[136] identified agonists of DR5 — a receptor for the tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) — that selectively induced apoptosis in cells overexpressing the *MYC* oncogene. Non-transformed primary cells were retrovirally infected to overexpress the *MYC* oncogene in order to understand the conditions in which MYC sensitizes cells to apoptosis. These cells were subjected to diverse stimuli. Many of these stimuli, such as DNA-damaging agents or serum starvation, decreased cell proliferation and cell cycle arrest. By contrast, TRAIL receptor activation decreased cell viability in cells that overexpressed *MYC* by inducing apoptosis. More specifically, using antagonistic monoclonal antibodies against different TRAIL death receptors, the authors showed that activation of DR5 resulted in *MYC*-selective apoptotic cell death. Treatment of *MYC*-overexpressing cells with an Aurora B kinase inhibitor, VX-680, initially kills cells by inducing apoptosis, but prolonged killing can occur via the autophagic cell death pathway[137]. These findings demonstrate that gain-of-function oncogenic mutations can be indirectly targeted by a synthetic lethality approach (TABLE 3).

Future perspectives and conclusions

The search for targeted therapies is a burgeoning field and synthetic lethal interactions represent a very promising means of selectively killing tumour cells. Furthermore, they could allow the exploitation of differences between tumour cells and normal cells that have previously been considered to be pharmacologically intractable. Future synthetic lethality screens could also be used to investigate mechanisms to exploit epigenetic phenomena, the tumour microenvironment and stromal–tumour interactions. Future conditional synthetic lethality screens should further investigate tumour hypoxia as a common physiological feature of solid tumours that can be targeted therapeutically. Other screens could focus on identifying genetic interactions to enhance radiotherapy or current cytotoxic chemotherapies. Overall, synthetic lethality screening could allow the field of oncology to shift its focus from 'first, hasten to help' back to the historical guiding medical principle of 'first, not to harm'.

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Acknowledgements
This work was supported by the following grants from the US National Cancer Institute: NCI-CA-67166 (A.J.G), NCI-CA-88480 (A.J.G) and NCI-CA-123823 (D.A.C). It was also supported by a grant from Action to Cure Kidney Cancer (A.J.G.). We apologize to colleagues whose work we failed to cite.

Competing interests statement
The authors declare competing financial interests: see Web version for details.

FURTHER INFORMATION
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