Synthetic Lethal Therapies for Cancer:
What's Next after PARP inhibitors for BRCA Mutated Cancers?

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Abstract

The genetic concept of synthetic lethality has now been validated clinically through the demonstrated efficacy of PARP inhibitors (PARPi) for the treatment of cancers in individuals with germline mutations in either *BRCA1* or *BRCA2*. Three different PARPi have now been approved for the treatment of *BRCA*-mutated ovarian cancer and one for *BRCA*-mutated breast cancer; these agents have also shown promising results in *BRCA*-mutated prostate cancer. Here we describe a number of other synthetic lethal interactions that have been discovered in human cancer. We will discuss some of the underlying principles that may enhance the likelihood of clinical efficacy and how new computational and experimental approaches are facilitating the discovery and validation of additional synthetic lethal interactions. Finally, we will make suggestions as to future directions and challenges facing this field.

(Main text 6500 words, 113 references)

1. Introduction

Synthetic lethality is a concept introduced almost 100 years ago by the fly geneticist Calvin Bridges[1] and named by Theodor Dobzhansky in 1946[2]. It describes the situation in a cell or organism whereby a defect in either one of two genes has little or no effect, but the combination of both gene defects results in sickness (synthetic sickness) or death (synthetic lethality)[3]. This concept had been long-studied by geneticists and developmental biologists but it was an article by Hartwell and colleagues[4], and subsequently highlighted by Kaelin[5], Brummelkamp, Bernards[6], and others, which fuelled interest in using the synthetic lethal concept to develop cancer therapeutics. synthetic lethal interactions are a form of context dependent essentiality[7], where a genetic alteration, such as a defect in a tumor suppressor gene (the context), causes a second gene to become essential for cell survival; drug inhibition of this second, synthetic lethal gene would in principle target tumour cells, but leave normal cells largely unaffected. As well as providing an approach that can be used to selectively target tumor cells and spare normal cells, application of the synthetic lethal principle also raises the prospect of precisely targeting subgroups of cancer that are defined by a known molecular change such as a driver mutation. As our understanding of the genetic alterations in cancer has developed, the synthetic lethal concept has been expanded to include a number of other related genetic concepts such as induced essentiality[8] and collateral sensitivity[9,10] (discussed below) and synthetic dosage lethality (SDL), a term that defines the situation when elevated activity of a gene (as opposed to loss) renders another gene essential.

The Poly(ADP-ribose) Polymerase 1 and 2 (PARP1 and PARP2) enzymes are important DNA damage sensors and signal transducers in the DNA Damage Response (DDR) that bind single strand DNA breaks (SSBs) and other types of DNA damage. Upon binding DNA, PARP1 and PARP2 post-translationally modify substrate proteins by synthesising poly(ADP-ribose) (PAR) chains (PARylation). Significant effort over many years has led to the discovery and development of small molecule inhibitors of PARP (PARPi), some of which have entered the clinic[11]. In 2005, two groups[12,13] discovered a synthetic lethal interaction between inhibition of PARP and *BRCA1* or *BRCA2* gene mutation.

BRCA1 and *BRCA2* are tumor suppressor genes that when heterozygously mutated in the germline confer considerably higher risks of several cancers including breast, ovarian, pancreatic

and prostate. BRCA1 and BRCA2 proteins are crucial to the repair of double strand DNA breaks by homologous recombination (HR)[11]. Tumors harbor loss of the wild-type *BRCA* allele and therefore carry a defect in HR. The synthetic lethal interaction between PARPi and loss of BRCA function is thought to be related to increased number of double strand DNA breaks or collapsed replication forks induced by PARP enzyme inhibition or trapping on DNA. Importantly, the magnitude of the synthetic lethal effect in laboratory models of BRCA mutation was substantial, an observation which encouraged early translation into clinic trials[11].

Initial observations in phase 1/1b and then phase 2 clinical trials demonstrated initial proof of concept of the validity of the approach, with multiple BRCA mutant cancer patients exhibiting profound and sustained responses to the PARPi olaparib (KuDOS/AstraZeneca)[14-16]. Subsequent trials confirmed these results and the United States Food and Drug Administration (FDA) has now approved three PARPi for clinical use: olaparib, rucaparib (Pfizer/Clovis) and niraparib (Merck/Tesaro) for *BRCA*-mutated ovarian cancer[11]. Olaparib has also now been approved by the FDA for the treatment of *BRCA*-mutated breast cancer (Table 1).

Although responses to PARPi in the BRCA-mutant group were profound enough to gain FDA approval, both *de novo* and acquired resistance to PARPi therapy is common. A number of mechanisms to explain this have been proposed, most of which center on the restoration of HR in tumor cells, either directly through reversion mutations in *BRCA1* or *BRCA2,* or indirectly via alterations in other components of the DDR[17]. It appears that, in common with other targeted therapies, PARPi treatment also results in Darwinian selection, favoring the survival and proliferation of tumor cell clones with *BRCA* gene reversion mutations above those that do not[18]. Considerable effort is now being directed at improving responses to PARPi, either through PARPi combination strategies[19] or through alternative targets in the *BRCA*-mutant tumor cells (see below).

The discovery and development of the first clinically approved synthetic lethal cancer therapy has fostered interest in the potential for exploiting other synthetic lethal interactions. In this review, illustrative recent examples of synthetic lethal interactions in diverse cellular pathways will be discussed with an emphasis on those that may eventually be clinically actionable. In addition, new technologies that may shape the discovery of additional synthetic lethal interactions will be described. Finally, new directions for the field will be suggested.

2. Synthetic Lethal Interactions by Cellular Pathway

(i) The DNA Damage Response Pathway (DDR)

The DDR is a set of highly connected molecular networks that maintain genomic integrity, thus preventing the transmission of altered genetic material to daughter cells and acting as a tumor suppressive barrier. For decades, gene perturbation studies in yeast have catalogued synthetic lethal interactions associated with genes involved in the DDR (for example[20,21]). One common feature of these studies has been the identification of multiple synthetic lethal interactions between pairs of DDR genes, an observation that likely reflects how multiple molecular processes interact to prevent the formation of what might be otherwise cytotoxic or fitness-impairing DNA lesions. The search for synthetic lethal interactions within the DDR has focussed largely on identifying: (a) additional synthetic lethal uses for PARPi; (b) alternative ways of synthetic lethal targeting *BRCA* mutant tumors that might supersede PARPi or overcome PARPi resistance; and (c) the targeting of non-BRCA and PARP related DDR synthetic lethal effects in tumors.

Additional synthetic lethal uses for PARPi. BRCAness is a term used to define a subset of tumors that lack germline *BRCA* gene mutations but share similar characteristics, in particular a defect in HR. As such they are *phenocopies* of germline BRCA mutant tumors[22]. Because of the defect in HR it might be expected that tumors with BRCAness might also show an synthetic lethal interaction with PARPi. In some respects, this has proved the case; defects in one of a number of tumor suppressor genes involved in HR and/or associated DNA repair pathways such as *ATM*, *ATR*, and *PALB2*, also cause sensitivity to PARPi in pre-clinical models[22,23]. Genetic screens have revealed additional genes defective in cancer, such as *CDK12*, *RAD51B* and *RAD51C*, that may also confer BRCAness[20]. Widespread sequencing of human tumors has revealed that significant proportions of high-grade serous ovarian cancers, advanced, castration-resistant, prostate cancer, pancreatic cancer and others harbor mutations in genes that could be classified as conferring BRCAness[22,24-26]. In addition, it seems possible that a combination of genetic, genomic and/or epigenetic defects in a single tumor cell could also cause an HR defect and BRCAness; however, the precise nature of these complex causes of BRCAness are still under investigation, as is their ability to predict significant clinical sensitivity to PARPi[22].

Alternative ways of synthetic lethal targeting *BRCA* mutant tumors. Given that resistance to PARPi is clinically important in *BRCA* mutant tumors there has been considerable effort in identifying additional *BRCA* synthetic lethal interactions that might either be superior to PARPi in

terms of therapeutic effect or that may overcome PARPi resistance. Two groups[27,28] have produced data suggesting that the low-fidelity DNA polymerase theta (Pol theta, encoded by the *POLQ* gene) may be a synthetic lethal target for the treatment of *BRCA1* mutated cancers. Pol theta is involved in a form of double strand DNA repair, known as microhomology-mediated end joining (MMEJ); this error-prone pathway requires PARP1. Because the survival of *BRCA1*-deficient cancer cells appears dependent on MMEJ, inhibition of Pol theta induces synthetic lethal. In part, this might be because Pol theta normally acts to inhibit HR (perhaps by sequestration of the DNA recombinase and BRCA2 interacting protein, RAD51) and therefore there may be an increased reliance on HR. In addition, silencing of Pol theta enhances the effects of PARPi or DNA cross-linking agents in HR-deficient ovarian cancer cells[23,24]. As a result of these studies, a number of companies are developing Pol theta inhibitors. The appropriate clinical context for the use of these agents, either superseding PARPi, used in combination with PARPi or used after PARPi resistance has emerged, remains to be determined.

RAD52 has a number of roles in DNA repair including playing a key role in the rescue of stalled replication forks[29]. Silencing of RAD52 is synthetic lethal with mutations in *BRCA1, BRCA2* or *PALB2*[30 31,32]. Although the exact mechanisms responsible for these synthetic lethal interactions remain to be fully understood, these observations have led to efforts to develop small molecule RAD52 inhibitors[33]. MUS81 endonuclease activity also appears to be required for replication fork progression in *BRCA2*-mutant cells. It has been proposed that inhibitors of MUS81 nuclease activity may have utility for the treatment of BRCA2-deficient cancers[34].

Patel and colleagues have shown that cells deficient in Fanconi Anemia (FANC) genes are sensitive to endogenous acetaldehyde produced as a consequence of cellular metabolism[35] . Recently, it has also been shown that defects in either BRCA1 or BRCA2 also sensitise cells to acetaldehyde. Endogenous acetaldehyde is removed through the action of Aldehyde Dehydrogenases (ALDHs); Tacconi et al. found that the drug disulfiram, an ALDH2 inhibitor, selectively inhibits the proliferation of BRCA1 and BRCA2 deficient cells[36]. Given that disulfiram in the form of Antabuse is in wide usage for the treatment of alcoholism, it seems possible that this work could be rapidly translated clinically.

Targeting other DDR defects in tumors. The success in exploiting synthetic lethality using PARPi has renewed interest in targeting other components of the DDR as synthetic lethal approaches to treating cancer. One of the most promising targets in this regard is ATR (Ataxia

Telangectasia Related), a protein kinase which is activated by RPA binding to single stranded (ss)DNA at stalled replication forks. CHK1, which is a critical regulator of the G_2/M and intra-S cell cycle checkpoints, is an important substrate of ATR. A related DDR kinase, ATM ((Ataxia Telangectasia Mutated) is also being targeted by drugs. Histone H2AX is a substrate of ATM and ATM phosphorylation of H2AX within histones flanking DNA double strand breaks facilitates the recruitment of additional DDR components. Another important substrate of ATM is CHK2 which has a role in the G_1/S cell cycle checkpoint. Potent inhibitors of ATR kinase activity in clinical development include VX-970 (Vertex Pharmaceuticals; now Merck KGA) and AZD6738 (AstraZeneca). A phase I trial of the ATM inhibitor AZD0156 (AstraZeneca) is ongoing (NCT02588105). These drugs are being investigated as single agents as well as in combination with chemotherapy, radiation, PARPi and immunotherapy. Biomarkers of response to ATR inhibitors are currently being explored and include ATM deficiency, DDR pathway mutations as well as genomic alterations that induce replicative stress including p53[37,38]. Mutation of the chromatin remodelling component, ARID1A, has also been suggested to mediate ATR inhibitor sensitivity[39] (see below). Clinical data is thus far limited but an anecdotal complete clinical response to an ATR inhibitor has been observed in a patient with metastatic *ATM*-deficient colorectal[40]. A number of other synthetic lethal interactions are being explored in the DDR space. One well validated such interaction is between RAD54B deficiency in colorectal cancer and silencing of the flap endonuclease FEN1[41].

(ii) Chromatin remodelling and epigenetic regulation

Mutations in genes that regulate chromatin structure and epigenetic regulation are present in up to 20% of all human malignancies[42]. Most commonly these mutations are in chromatin remodelling tumor suppressor genes but gain of function mutations also occur. For example, the SWI/SNF BAF and PBAF chromatin remodelling complexes are composed of more than a dozen protein subunits, many of which are well-established tumour suppressor proteins. These SWI/SNF complexes mediate the mobilisation of nucleosomes along DNA, allowing the regulation of gene expression and facilitating DNA replication and repair processes. A number of synthetic lethal interactions have now been described for deficiencies in SWI/SNF-complex components, some of which are being tested in clinical trials[42].

In what may be a general phenomenon related to protein complexes and synthetic lethality (see later), loss of some SWI/SNF subunits is synthetic lethal with depletion of other SWI/SNF subunits. For example, SNF5 (SMARCB1) and the ATPase BRG1 are synthetic lethal; the inactivation of Brg1 abrogates tumorigenesis in Snf5 deficient mice. Human SWI/SNF complexes contain one of two core ATPases, BRG1 (SMARCA4) and BRM (SMARCA2) which are mutually exclusive. *BRG1* is often mutated in cancers, including those of the lung, pancreas and brain. Several groups showed that *BRG1* and *BRM* are synthetic lethal, both in cell lines and *in vivo,* due to absence of ATPase activity in the SWI/SNF complex[43-46]. This is one such example of defects in paralogs causing synthetic lethal (see later and **Figure 1**).

SNF5 is a component of the SWI/SNF complex which is frequently mutated in childhood malignant rhabdoid tumors as well as other cancers. One such synthetic lethal interaction showing particular promise is between SNF5 deficiency and inhibition of the polycomb complex histone methyltransferase EZH2. EZH2 induces epigenetic silencing of the *CDKN2A* tumor suppressor gene when SNF5 is deficient. Upon inhibition of EZH2, *CDKN2A* is reactivated suppressing proliferation. Whether EZH2 inhibition shows clinical efficacy in SNF-deficient cancers is now being tested in clinical trials. EZH2 inhibition[47,48] also shows synthetic lethal with SWI/SNF components ARID1A, BRG1 (SMARCA4) and PBRM1[49].

Deleterious mutations in *ARID1A*, which encodes a DNA binding subunit of the BAF complex, are present in multiple human cancers including of the endometrium (21%[50]), liver (10%[51]) stomach (21%[52]) and other sites. Over half of the hard to treat ovarian clear cell carcinomas carry a mutation in *ARID1A.* Because of the prevalence of *ARID1A* mutations, a number of investigators have identified genetic and chemical synthetic lethal partners of *ARID1A*. Project Achilles (described below) identified defects in the paralogous DNA binding BAF subunit, *ARID1B,* as being synthetic lethal with ARID1A defects; absence of both paralogs leads to destabilisation of the SWI/SNF complex[53] (**Figure 1**). ARID1A has been suggested to facilitate maintenance of genome stability through a role in the repair of DNA breaks by homologous recombination repair. As such ARID1A-deficient cells have been reported as being modestly sensitive to PARPi treatment *in vitro*[54,55]. In addition, ARID1A deficiency has been shown to be synthetic lethal with inhibition of PI3K/AKT[56] and also to result in sensitivity to the kinase inhibitor dasatinib[57]. Recently, a profound sensitivity of ARID1A deficient cells to ATR inhibitors has been described[55]. Mechanistically, ARID1A-deficiency results in DNA processing defects during mitosis, which cause an increased reliance upon ATR function. Clinical trials to test this hypothesis are

underway. Models of ovarian clear cell carcinoma with defects in ARID1A have also been shown to be reliant upon the histone deacetylase protein, HDAC6[58]. Loss of the repressive effect of ARID1A on *HDAC6* transcription allows HDAC6 to deacetylate p53, reducing its pro-apoptotic function, an effect that is apparent in pre-clinical models using the clinical HDAC inhibitor, ACY1215[58].

Synthetic lethal relationships have been described in epigenetic regulatory pathways beyond SWI/SNF. *SETD2*, encodes a histone methyltransferase that catalyses the production of Histone H3K36me3, which is associated with heterochromatin[59]. Loss of function mutations in *SETD2* are common in clear cell renal carcinomas. Inhibition of the cell cycle checkpoint kinase WEE1 has been shown to be synthetic lethal with *SETD2* mutation. WEE1 inhibition in SETD2 mutant cells appears to result in reduced levels of the ribonucleotide reductase subunit RRM2 reduction leading to dNTP depletion and S-phase arrest[60].

(iii) Signal Transduction Pathways

Aberrations in cell signalling occur frequently in human cancers. In particular, the Ras family of oncogenes (*Kras, Nras*, and *Hras*) are the most common genetic drivers, being activated by mutation in approximately 20% of cancers. Despite considerable efforts over many decades, direct inhibitors of Ras are not yet available clinically, although there are some signs that novel approaches to drug discovery may be more fruitful in this regard (e.g.[61]). Because of this, indirect approaches such as the use of synthetic lethality have been explored. Both mechanism driven and screening approaches have been employed. Although the delineation of robust Ras synthetic lethal effects has not been straightforward, a number of lessons have been learned that may allow a path towards identifying clinically actionable effects. Furthermore, the considerable effort expended in searching for Ras synthetic lethal effects has established principles that may assist the discovery of robust synthetic lethal effects in other systems.

Many mutant Ras synthetic lethal screens have been published. For example, an early short hairpin RNA (shRNA) screen of the kinome identified the TANK-binding kinase 1 (TBK1) as a Kras synthetic lethal target[62]. This appeared to operate via TBK1 activation of the NF-kB pathway, a requirement for survival of Kras-mutant driven lung cancer cells. Another screen identified the kinase STK33 as being a synthetic lethal partner of mutant Ras. However, subsequent attempts to validate STK33 have led some to conclude that the synthetic lethal interaction was not widely applicable and might be private to specific model systems[63 64]. In general, there has been little

overlap in the synthetic lethal effects identified in different synthetic lethal screens. There are a number of possible explanations for this that have been thoughtfully discussed by Downward[65]. These include the heterogeneity of the cell systems (genetic background, cellular state, histology) used, whether the effects operate in 2D tissue culture rather than *in vivo*; differences between individual RAS mutations and methodological differences in the statistical analysis of high throughput screens. The general principle established in this work however, is that many of the synthetic lethal effects identified in individual *Kras* mutant model systems might be relatively private to these systems; these private events are readily reversed when assessed in a different *Kras* mutant context (i.e. a different model system), and are thus unlikely to be robust in the face of the considerable molecular and phenotypic heterogeneity seen in human cancers[3].

Recently, Sabatini and colleagues[66] have performed genome-wide CRISPR/Cas9 screens in 14 Acute Myeloid Leukemia (AML) cell lines. The comparison of six *KRAS/NRAS* mutant lines with six wild-type lines allowed the identification of a small number of synthetic lethal genes which are involved in RAS processing (e.g. *RCEI* and *ICMT*) and MAPK signalling (e.g. *RAF1*, *SHOC2*). These same synthetic lethal effects were reconfirmed in isogenic matched Ba/F3 cells with a *NRAS* mutation. These results suggest that at least in AML, there are relatively few RAS synthetic lethal partners and that these are restricted to RAS processing or MAPK signalling. That only a few *bona fide* RAS synthetic lethal effects were discovered and that these can be histology-specific, may go some way to explaining why large-scale screens of heterogeneous cell lines from multiple different cancer types, have failed to identify common RAS synthetic lethal genes.

A number of other Ras synthetic lethal interactions (reviewed recently[67]) have been described with genes from the DDR[68] (for example, ATR[69]), cell cycle (for example, CHK1)[70,71], the proteasome and proteolytic stress (e.g. through HSP90 inhibition)[72] as well as with transcription factors, such as GATA2[73,74]. It remains to be seen whether these will prove to have clinical utility.

Synthetic lethal approaches have been used to investigate signal transduction genes other than RAS. For example, using a mutual exclusivity approach DePinho and colleagues[75] discovered chromatin helicase DNA-binding factor CHD1 depletion as being potentially selectively lethal to PTEN-deficient prostate and breast cancers. PTEN normally promotes CHD1 degradation via the β-TrCP-mediated ubiquitination–proteasome pathway. The absence of PTEN results in stabilization of CHD1, leading to activation of pro-tumorigenic TNF–NF-κB signalling.

(iv) Metabolism

Somatic mutations in cancer have been identified in genes involved in energy metabolism opening up the possibility of targeting the rewired metabolic state via synthetic lethal approaches. *FH* (*Fumarate hydrolase*) germline mutations are associated with hereditary leiomyomatosis and renal cell carcinoma. FH deficiency leads to the accumulation of fumarate, which activates hypoxia-inducible factors (HIFs) in the absence of hypoxia. However, it was unclear how cells survive in the absence of functional tricarboxylic acid (TCA or Krebs') cycle. Frezza *et al.*[76] used computational modelling and metabolomic profiling to identify a metabolic alteration that occurs as a consequence of FH deficiency. Truncation of the TCA cycle resulted in movement of glutamine-derived carbon atoms into the heme biosynthesis and degradation pathway. As a result, FH1-deficient cells can use accumulated TCA cycle metabolites to allow sufficient mitochondrial NADH production. However, as a result, silencing of Haem Oxygenase 1, or inhibition of the enzyme with Zinc protoporphyrin, resulted in selective growth impairment of Fh1-deficient cells compared to control cells.

Mutations in the key respiratory enzyme Succinate Dehydrogenase (SDH complex) are present in the rare tumors pheochromocytoma, paraganglioma and gastro-intestinal stromal tumors[77]. Mechanistic analysis suggests that mutations in SDH complex genes result in a truncated TCA cycle which causes a defect in aspartate production which is normally required for nucleotide biosynthesis. As an alternative, SDH mutant tumors use pyruvate and pyruvate carboxylase to sustain aspartate biosynthesis. As a consequence, SDH mutant tumors are hypersensitive to pyruvate carboxylase silencing, while non-transformed cells are able to use the canonical pathway for aspartate biosynthesis[78].

Gain of function mutations in two related Isocitrate Dehydrogenases (IDH1 and IDH2) occur in gliomas and Acute Myeloid Leukemias (AML)[79]. Mutations in these enzymes lead to the neomorphic production of the oncometabolite (*R*)-2-hydroxyglutarate (2-HG). Inhibitors of mutant IDH1/2 have been developed and have shown efficacy in the clinic [79]. In addition, studies have been performed investigating synthetic lethal approaches to treating IDH1/2 mutant cancers. IDH1/2 mutant cells are sensitive to PARPi[80]; 2-Hydroxyglutarate produced by neomorphic IDH mutations appears to suppress DNA repair by HR and hence sensitises cells to PARPi. Moreover, synthetic lethal relationships to IDH1/2 with the apoptotic proteins Bcl2 in AML and BclXL in glioma have been described [81,82].

The von Hippel–Lindau tumor suppressor gene (VHL), is commonly mutated or promoter hypermethylated in clear cell renal cell carcinoma[83]. VHL encodes an oxygen-dependent ubiquitin ligase that regulates the degradation of the hypoxia-inducible factor 1α (HIF1α) transcription factor. In the presence of oxygen, HIF1α is hydroxylated on proline residues by EglN prolyl hydroxylases and subsequently ubiquitinated by VHL. Loss of VHL activity results in dysregulated HIF1α activity which drives the development of renal cell carcinoma[83]. A number of synthetic lethal interactions with VHL loss have been described. An siRNA screen identified the histone methyltransferase EZH1 as a potential synthetic lethal partner of VHL loss; this interaction was validated by pharmacological inhibition of EZH1. In addition, chemical screens identified inhibitors of autophagy, GLUT1 and Rho-associated kinase 1 as potential synthetic lethal therapies for VHL-related cancers[84-86].

When tumor suppressor genes are deleted in cancer other putative passenger genes may also be co-deleted which result in potential vulnerabilities; the loss of such genes has been termed collateral lethality/sensitivity[9,10]. Three groups discovered that deletion of the *MTAP* gene confers enhanced dependence on the PRMT5 arginine methyltransferase[87-89]. MTAP is adjacent in the genome to the tumor suppressor gene *CDKN2A* (p16). *CDKN2A* is commonly homozygously deleted in cancer and MTAP is frequently co-deleted with *CDKN2A* (~15% of all cancers and >50% of glioblastoma multiforme). MTAP encodes an enzyme which cleaves methylthioadenosine (MTA) generating precursors for methionine and adenine synthesis. The absence of MTAP leads to an accumulation of MTA in the cell which leads to partial inhibition of PRMT5. As a consequence, cancers with loss of MTAP are more sensitive to inhibition of PRMT5[87-89] and number of companies are now developing PRMT5 inhibitors.

ENO1, encoding the important glycolytic enzyme, enolase, is often homozygously deleted at chromosome 1p36, alongside multiple tumor suppressor genes. In the absence of enolase, cells cannot produce ATP because of defective glycolysis. The fact that such a deletion is not lethal to the cell may be due to the existence of a paralog of *ENO1* called *ENO2*. DePinho and colleagues[10] showed that shRNA depletion or chemical inhibition of ENO2 was selectively lethal to glioma cells carrying an *ENO1* deletion suggesting a targetable. synthetic lethal-type interaction. Similarly, genomic deletion of Malic enzyme 2 in pancreas cancer also confers collateral lethal dependency on Malic enzyme 3[90].

(v) Synthetic lethal interactions with other major cancer-related genes

Despite the considerable success in developing targeted therapy, for mutated kinases in cancer there has been little progress in developing therapies for many of the major genes that are mutated or overexpressed in cancer such as Myc, Rb and P53. These remain significant targets for synthetic lethal approaches.

Myc: The Myc oncogene is dysregulated in a significant proportion of human cancers. There are three Myc family members c-, L-, and N-Myc, all of which have been linked to tumor pathogenesis and progression. The Myc proteins are transcription factors that regulate multiple cellular functions including proliferation, growth and metabolism[91]. There is considerable evidence that Myc is potentially an extremely important therapeutic target. However, as a transcription factor, Myc has proved thus far impossible to target directly. Therefore, a number of attempts have been made to target Myc indirectly using synthetic lethal approaches[92,93].

Given the role of Myc proteins in cellular proliferation, considerable attention has been paid to synthetic lethal interactions with cell cycle regulatory components; for example, small molecule inhibition of CDK2 is synthetic lethal with Myc overexpression[94]. In addition, CDK1 inhibition using the inhibitor purvalanol A induces apoptosis in cells overexpressing Myc, prolongs survival in mouse myc–driven lymphoma models[95]. An additional link to apoptosis was provided by the finding that TRAIL and DR5-agonists were synthetic lethal with dysregulated Myc[96].

A number of other Myc synthetic lethal interactions have been described such as SAE1/2, an enzyme involved in SUMOylation which was identified in a MYC synthetic lethal genome-wide RNAi screen. In Myc driven cells, depletion of SAE1/2 caused mitotic catastrophe in Myc hyperactive cells[97]. PIM1 kinase was identified in MYC synthetic lethal kinome RNAi screen and appears to act inhibiting the oncogenic transcriptional activity of MYC and restoring the activity of the cell cycle inhibitor, p27[98]. As PIM1 kinase inhibitors are available, this observation could be readily tested clinically. Other Myc synthetic lethal interactions with genes involved in pathways such as transcription[99], RNA splicing[100] and metabolism [101] amongst others.

Rb: *RB1 (Rb)* is commonly lost by mutation or transcriptional silencing in the childhood eye cancer, retinoblastoma, breast cancer, prostate, lung cancer, sarcomas, and glioblastoma [102]. The canonical role of Rb is in cell-cycle control and proliferation[103,104], where Rb, together with D-type

Cyclins, Cyclin-dependant kinases and inhibitors, Rb pocket proteins and the E2F family of transcription factors, control the progression of cells through the G_1 restriction checkpoint. A number of chemogenetic Rb synthetic lethal interactions have been identified, including topoisomerase poisons, such as etoposide; Rb is thought to mediate the processing and repair of DNA damage caused by these agents by binding both Type II topoisomerases and BRCA1 and recruiting them to the site of DNA damage[105]. A number of synthetic lethal gene targets associated with Rb defects have also been identified. Simultaneous inactivation of *Rb* and the *Tsc2* in *Drosophila* causes synthetic lethality, an effect conserved in human tumour cells[106]. TSC2, together with TSC1, forms a heterodimer that controls mTOR (mechanistic target of rapamycin) signalling via TORC1; the synthetic lethal between Rb and TSC2 appears to be dependent upon dysregulation of the TORC1 effector S6-kinase and the Rb-dependent transcription factor E2F1[106]. Similarly, upregulated Wnt signalling, caused for example by loss of *APC* tumour suppressor gene function, also causes Rb synthetic lethal via TORC1 dysregulation[107]. Large scale shRNA screens in human tumour cell lines demonstrated the dependency of Rb defective tumour cells upon E2F-family transcription factors including E2F3[108,109]. This might relate to the roles that some E2F transcription factors have in minimising replication stress and DNA damage in cells with Rb defects[110,111]. Finally, combined defects in Rb and p53 impart a dependency upon the RNase III enzyme Dicer1 perhaps related to the oncogenic miR-17-92 cluster[112].

p53: p53 is one the most commonly mutated tumor suppressor genes; p53 itself or regulators of p53 activity are dysfunctional in most human cancers[113]. The p53 gene encodes a transcription factor activated by multiple cellular stresses that is a major regulator of DNA repair, cell cycle arrest apoptosis and metabolism[114]. Moreover, loss of p53 function affects the response to many cytotoxic chemotherapies and is associated with poor survival. These observations have led to many efforts to restore p53 function or to inhibit aberrant p53 signalling in cancers. In addition, multiple synthetic lethal approaches to targeting p53 mutant tumors have been discovered in many cellular pathways including the DDR (see section 2i), metabolism and cell cycle regulation; the area of synthetic lethal interactions with P53 has recently been extensively reviewed[115].

3. Approaches to discover and validate synthetic lethal and CS interactions

Cancer derived cell lines cultured *in vitro* on plastic have been the mainstay of experimental systems to define new synthetic lethal relationships. For example, genome-wide RNA interference screens in large panels of cancer derived cell lines, where the dependency upon multiple distinct

genes is established, has allowed a first draft of synthetic lethal effects that operate in cancer cells to be delineated [108,109,116-118]. This large-scale mapping of synthetic lethal relationships in human cancer cells has also facilitated a more refined classification of synthetic lethal effects. For example, an analysis of synthetic lethal effects associated with 800 genes in 400 cancer cell lines, identified three broad, and sometimes overlapping, classes of synthetic lethal effect[109]: (a) *pathway synthetic lethal effects*, where synthetic lethal partners act in either parallel pathways or within the same pathway to modulate a process that is essential in tumor cells; an example is the synthetic lethal between the tumor suppressor APC and β-catenin, both of which modulate oncogenic Wnt signalling; (b) *paralog effects*, where synthetic lethal partners are paralogs that still retain at least one overlapping function that is essential in tumor cells, e.g. the cyclin dependent kinases CDK4 and CDK6, or the SWI/SNF proteins ARID1A and ARID1B; and (c) collateral lethality effects, as described earlier. Similarly, other classifications of synthetic lethal have been proposed that include the overlapping concepts of protective essential genes[7] and induced essential effects[8], both of which describe the scenario where an alteration in one gene is deleterious unless mitigated by the activity of a second gene; inactivation of this second gene leads to synthetic lethality. This might be highly relevant in cancer, where driver alterations (e.g. oncogene activation or tumour suppressor gene loss) impart an eventual fitness advantage upon cells but might also cause deleterious effects that if not kept in check, could result in cell death. For example, replication fork stress caused by oncogenes may be tumor promoting (for example, by promoting DNA mutations) but might also impart a heightened requirement for proteins, such as ATR, that prevent the deleterious effects of excessive fork stress, an effect that could be exploited with clinical ATR inhibitors. Defining the different modes by which synthetic lethal effects operate could, in principle, allow novel synthetic lethal interactions to be predicted, rather than identified empirically.

Other approaches, both computational and experimental, are also augmenting the search for new synthetic lethal interactions. For example, the delineation of genome-wide protein–protein interaction (PPI) maps in model organisms has led to the observation that essential proteins tend to have a higher number of interactions than those that are less essential[7]; in principle, applying a similar analysis to understand the interactome of synthetic lethal target proteins could facilitate the prediction of new synthetic lethal effects. Furthermore, a recent analysis of tumour profiling datasets suggests that loss of individual protein complex components in tumors (for example via gene deletion), often results in a partial or complete loss of other parts of the protein complex from the tumour[119]. In some cases, further inhibition of these collaterally-

lost complex subunits results in synthetic lethal[119], suggesting that an *in silico* analysis of tumor proteomes could be used to predict synthetic lethal effects.

At the experimental level, advances in gene perturbation technologies will likely enhance the ability to empirically identify and validate synthetic lethal interactions. Whilst much of the focus over the past decade has been on exploiting RNA interference, transposon mutagenesis and chemical mutagenesis of genomes to identify synthetic lethal effects, gene editing by CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) approaches has gained widespread application in this regard. More recently, modifications of the CRISPR system have become available so that genes can be transcriptionally silenced (CRISPRi) or activated (CRISPRa)[120]. These methods can be used in combination to define genetic interactions on a large scale. Moreover, CRISPRi has been combined with single cell sequencing in a technique called PERTURB-Seq[121] to uncover new components of pathways as well as the targets of drugs. CRISPR systems adapted to mutate or alter the expression of more than one gene are ideally suited to the definition of genetic interaction networks and may allow the discovery of higher order interactions.

4.Conclusions and Future Prospects

The success of the discovery and development of parpi as an synthetic lethal therapy for *BRCA1* and *BRCA2*-mutated cancers has driven research into better understanding of genetic interactions between cancer genes and potential therapeutic targets. as well as hypothesis-driven approaches, large scale experimental and computational methodologies are being used to discover cancer specific synthetic lethal interactions at an unprecedented rate. however, parpi remain the only fda approved synthetic lethal therapy and many of the major genetically altered genes in cancer such as p53 and rb remain undrugged. it is important, therefore, to consider what will be required to discover and develop additional such therapies.

In considering whether a given synthetic lethal interaction might eventually deliver clinical benefit it is critical to take the magnitude of the synthetic lethal effect into account. in terms of the size of the synthetic lethal effect the parpi/brca interaction is substantial being >100-fold depending on the system. Some of the synthetic lethal interactions described thus far may be statistically significant and even biologically meaningful but are too small to translate into efficacy in the clinic. Discovery and validation strategies should emphasise substantial synthetic lethal effects. Likely

better screening technologies linked to improved computational analysis will yield more suitable synthetic lethal interactions.

Some synthetic lethal interactions, as exemplified by many of those in the RAS field, are histology, cell type or genetic background dependent ie private. These “soft” synthetic lethal effect need a distinct development strategy compared to “hard” effects such as the PARPi/BRCA interaction which are more resistant to this heterogeneity. The choice of systems for discovery and development is also important. For many tumor suppressor genes there are insufficient numbers of cell lines available that carry specific genetic lesions especially when heterogeneity is taken into account. This limits evaluation of histology dependent effects.

One clear challenge to the field is how methods might be developed that allow robust synthetic lethal effects (i.e. those that are relatively resilient to other molecular changes or plasticity) to be predicted from first principles; at present the robustness of synthetic lethal effects is critical if effective treatments are to be developed but is still largely determined by empirical means (e.g. assessment in multiple model systems) which are often time consuming and expensive. it seems reasonable to think that the ability to discriminate robust synthetic lethal effects from those that are less robust might emerge from learning the characteristics of known, robust, synthetic lethals. some of these characteristics might emerge from the further analysis of existing synthetic lethal screens (e.g. large-scale shrna or crispr-cas9 screens in cancer cell lines) or even by assessing whether some of the predictable characteristics of essential genes in model organisms[7] apply to cancer cells. moreover, screens and subsequent validation in more biologically appropriate systems such as 3d organoid culture or *in vivo* in mice might yield more robust synthetic lethal effects.

An underexploited aspect of the synthetic lethal principle is the simultaneous exploitation of multiple distinct synthetic lethal effects to uncover novel combination therapies. Most solid tumours are driven by a number of truncal driver mutations, offering the possibility of rationally designing combination therapies that target multiple distinct driver effects at the same time. For example, the co-occurrence of *APC* tumour suppressor mutations with *KRAS* mutations in some tumours might predict response to Wnt pathway inhibitors that are synthetic lethal with the *APC* defect as well as MAP-kinase signal transduction inhibitors that target the *KRAS* addiction. Likewise, in animal models of triple negative breast cancer that possess both *Brca1* gene defects and also *Fgfr2*-oncogenic fusions, the combination of a PARP inhibitor, targeting the Brca1 defect,

and a FGFR inhibitor, targeting the Fgfr2 fusion, is more effective than either agent alone[122]. Such **combination synthetic lethal approaches** are not conceptually complex but might deliver comparable, if not better, results that combination therapies that act synergistically on a single driver mutation, so long as the two drugs used have distinct mechanisms of action and are therefore less likely to be impacted by singular mechanisms of drug resistance. Already there is some understanding that many of the drug combination approaches that have empirically been found to be effective in treating cancer work by combining distinct mechanisms of action[123]; perhaps the task ahead is to rationally design new approaches that exploit this effect, by first identifying synthetic lethal interactions with individual driver gene defects.

A separate extension of the synthetic lethal principle might be to identify **higher order synthetic lethal effects**. Rather than single cancer driver genes being synthetic lethal with inhibition of a target (e.g. inhibition of A is synthetic lethal with a defect in B), inhibition of a combination of targets is required to elicit a synthetic lethal effect (e.g. inhibition of A, plus inhibition of B, is synthetic lethal with a defect in C). There seems no *a priori* reason why such higher order synthetic lethal interactions might not be as prevalent as classical synthetic lethal effects. Indeed, many of the proposed drug combination strategies in cancer might already exploit this concept, albeit not by design; such higher order effects probably work by simultaneously targeting a driver gene effect by synthetic lethality as well as any synthetic rescue effect that would otherwise reverse the primary synthetic lethality.

In conclusion, therefore, the approval of PARPi has driven excitement about the prospects of further clinically effect synthetic lethal therapies. A number of already discovered additional synthetic lethal interactions are showing promise and with the advent of powerful new technologies many more are likely to be revealed.

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Table 1. PARP Inhibitors that have received FDA regulatory approval for clinical use

Agent	Company	FDA Approval(s)
Olaparib (Lynparza)	AstraZeneca (formerly KuDOS)	**Ovarian Cancer:** for the maintenance treatment of adult patients with recurrent epithelial ovarian, fallopian tube or primary peritoneal cancer, who are in a complete or partial response to platinum-based chemotherapy. **Ovarian Cancer:** for the treatment of adult patients with deleterious or suspected deleterious germline *BRCA*-mutated advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy. Patients are selected for therapy based on an FDA-approved companion diagnostic that identifies deleterious germline *BRCA1* or *BRCA2* gene mutations (Myraid, BRACAnalysis CDx test). **Breast cancer:** in patients with deleterious or suspected deleterious germline *BRCA*-mutated, human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer who have been treated with chemotherapy in the neoadjuvant, adjuvant or metastatic setting. Patients with hormone receptor (HR) positive breast cancer should have been treated with a prior endocrine therapy or be considered

		inappropriate for endocrine therapy. Patients are selected for therapy based on an FDA-approved companion diagnostic that identifies deleterious germline *BRCA1* or *BRCA2* gene mutations (Myraid, BRACAnalysis CDx test).
Rucaparib (Rubraca)	Clovis (formerly Pfizer)	**Ovarian Cancer:** monotherapy for the treatment of patients with deleterious *BRCA* mutation (germline and/or somatic) associated advanced ovarian cancer who have been treated with two or more chemotherapies. Patients are selected for therapy based on an FDA-approved companion diagnostic that identifies deleterious germline *BRCA1* or *BRCA2* gene mutations (FoundationFocus CDxBRCA test).
Niraparib (Zejula)	Tesaro (formerly Merck)	**Ovarian Cancer:** for the maintenance treatment of adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy

Figure Legends

Fig 1. Pathway based synthetic lethality – synthetic lethality between parallel pathways acting on a process that is essential in tumor cells. An example of the synthetic lethal interaction between BRCA1 and POLQ is shown (refs 27,28).

Fig 2. Pathway based synthetic lethal - synthetic lethality between multiple components in a pathway that is essential in tumor cells. Interactions in the Wnt pathway are shown.

Fig. 3. Paralog based synthetic lethal – synthetic lethality between two paralogs. An example of the synthetic lethal interaction between SWI/SNF components ARID1A and ARID1B are shown (ref 53).

Fig. 4. Collateral synthetic lethal via loss of genetic material– the collateral loss of genetic material (deleted material indicated by blue shading) linked to a tumour suppressor gene causes a haploinsufficiency effect. In this example genetic material carrying the MTAP gene is lost when the cancer driver CDKN2A is deleted. Loss of MTAP is synthetic lethal with PRMT5 inhibition (refs 87-89).

Fig. 5. Collateral synthetic lethal via complex collapse – loss of one protein subunit causes complex collapse and new vulnerability (ref 119).

Competing Statement

AA is or has been:

Consultant for:
Genentech
AtlasMDX
Third Rock Ventures
Pfizer
Sun Pharma
Bluestar

Grant/Research support from:
Sun Pharma

I am a co-founder of Tango Therapeutics.

I hold patents on the use of PARP inhibitors jointly with AstraZeneca which I have benefitted financially (and may do so in the future) through the Institute of Cancer Research Rewards to Inventors Scheme

CJL is or has been:

Consultant for:

Third Rock Ventures
Astra Zeneca
Sun Pharma
GLG
Guidepoint
Vertex

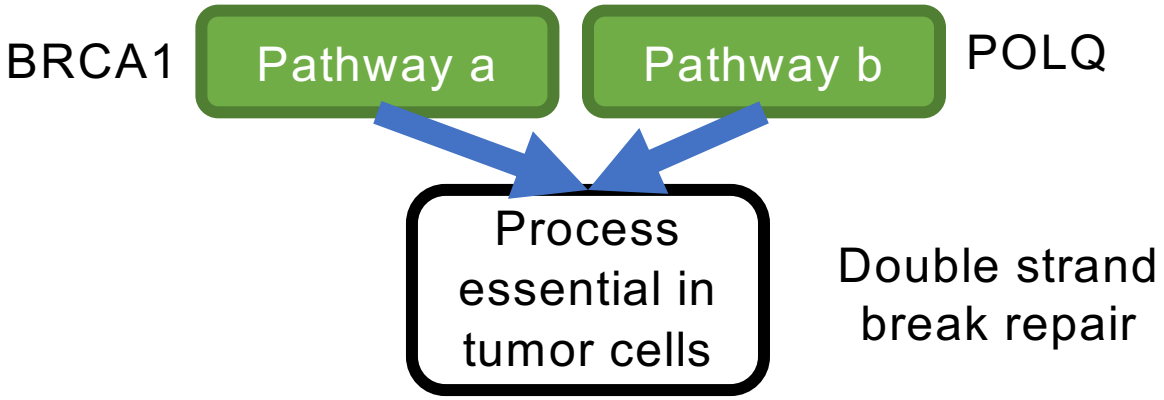
Grant/Research support from:
Astra Zeneca
Merck KGaA

I hold patents on the use of DNA repair inhibitors and stand to gain from their use as part of the Institute of Cancer Research "Rewards to Inventors Scheme".

Acknowledgements

AA thanks Susan G Komen for the Cure, The Breast Cancer Research Foundation and the BRCA Foundation for financial support.

CJL thanks Cancer Research UK and Breast Cancer Now for financial support.



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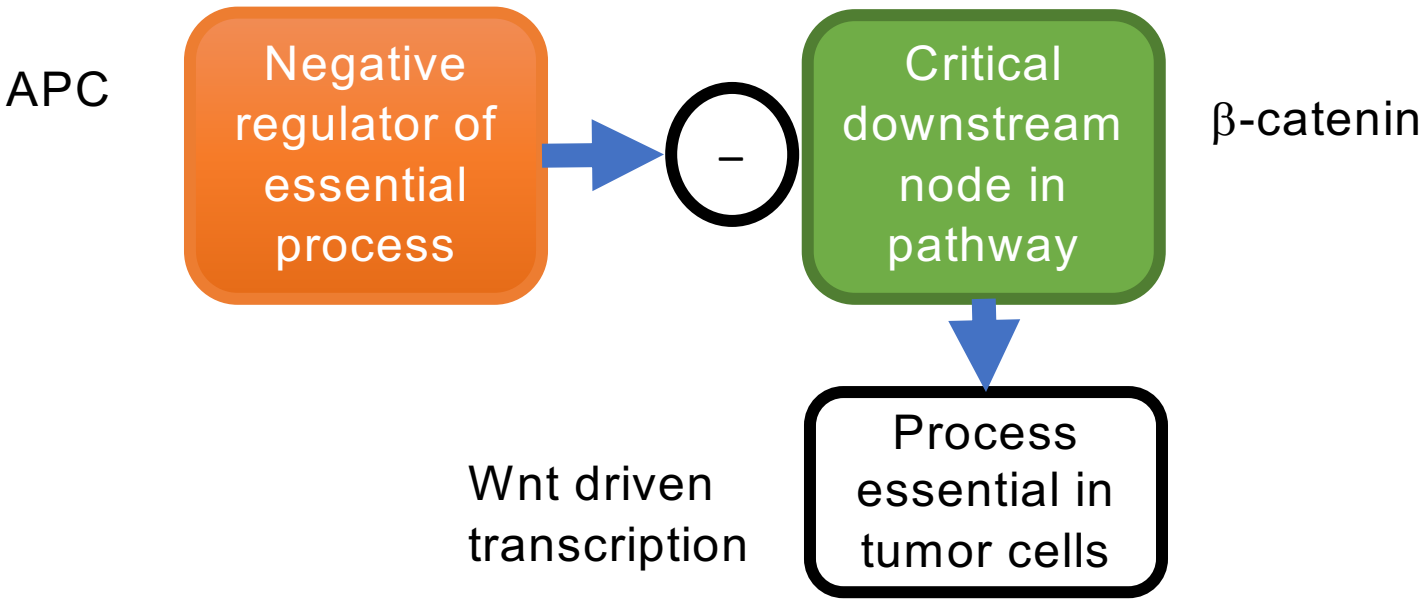


Fig 2. Pathway based synthetic lethal - synthetic lethality between multiple components in a pathway that is essential in tumor cells. Interactions in the Wnt pathway are shown.

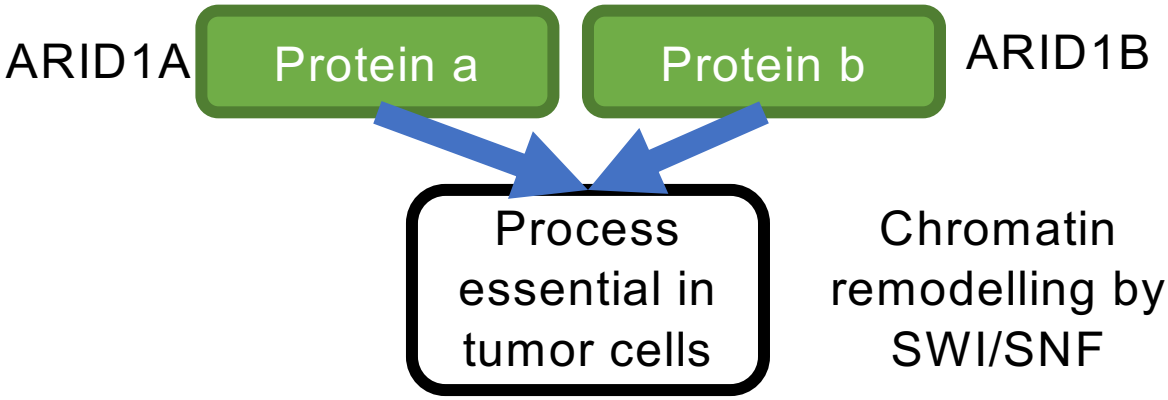
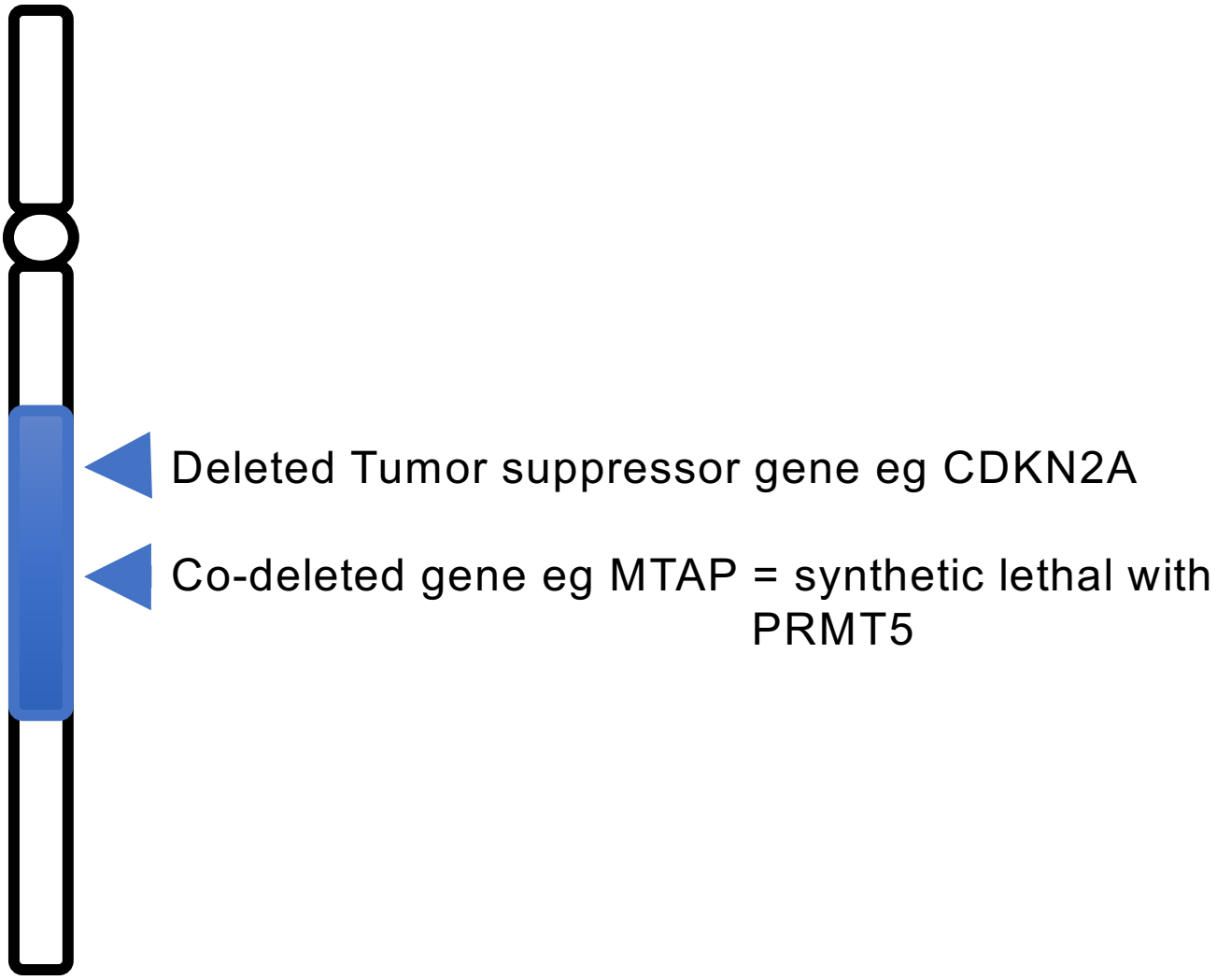


Fig. 3. Paralog based synthetic lethal – synthetic lethality between two paralogs. An example of the synthetic lethal interaction between SWI/SNF components ARID1A and ARID1B are shown (ref 53).



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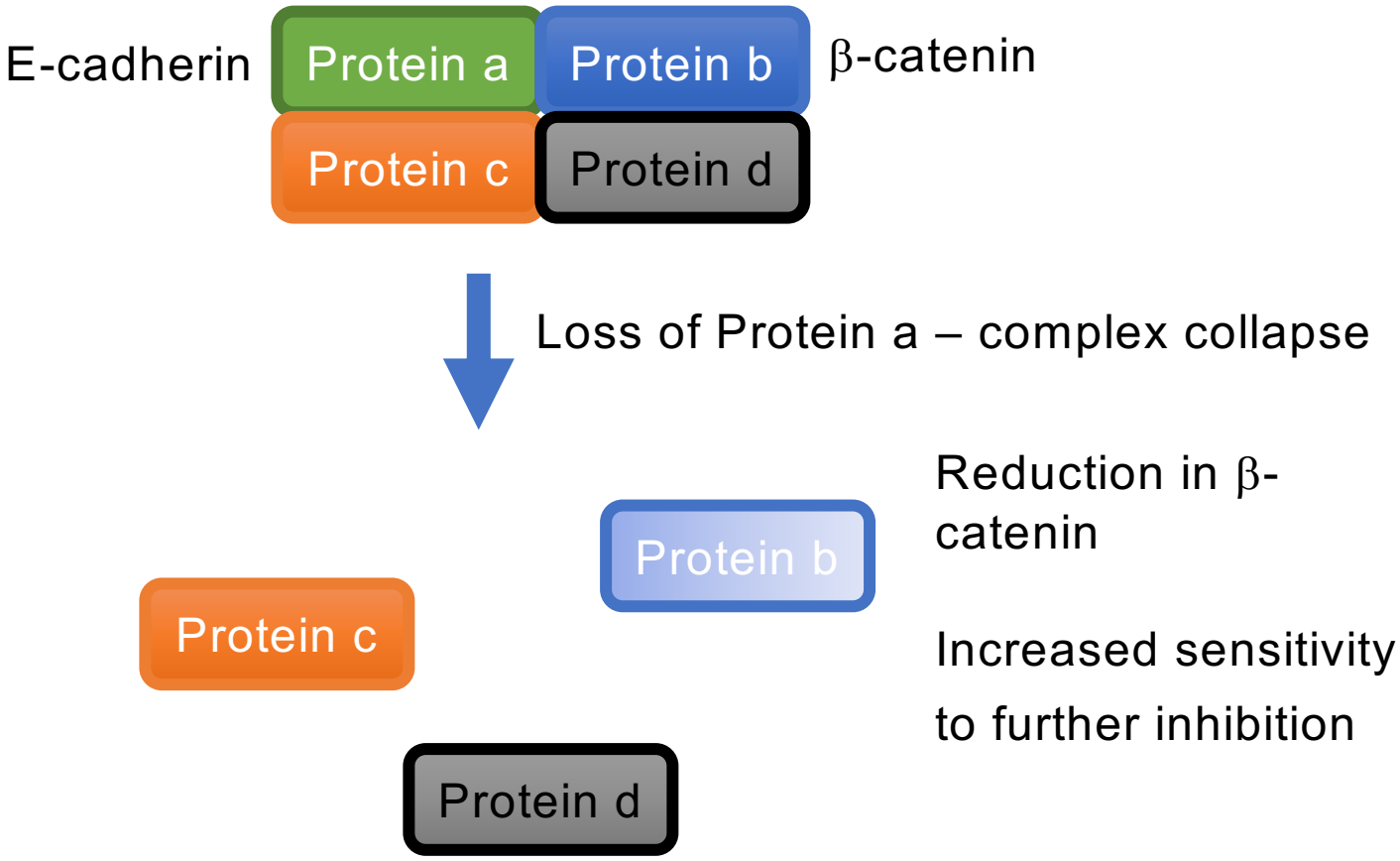

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