Synthetic Lethal Networks for Precision Oncology: Promises and Pitfalls

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Title: Synthetic Lethal Networks for Precision Oncology: Promises and Pitfalls

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Highlights:
- Synthetic lethal interactions have the potential to greatly expand the scope of precision oncology
- New technologies allow for high-throughput screens to identify new synthetic lethal interactions
- Consideration of cellular context will be critical when designing synthetic lethal cancer therapies

Keywords: Synthetic Lethal, Cancer Genomics, Precision Medicine, Systems Biology, Genetic Interaction

Abbreviations:
tumor suppressor gene (TSG)
homologous recombination (HR)
artificial intelligence (AI)

Graphical abstract

Abstract: Synthetic lethal interactions, in which the simultaneous loss-of-function of two genes produces a lethal phenotype, are being explored as a means to therapeutically exploit cancer-specific vulnerabilities and expand the scope of precision oncology. Currently, three FDA approved drugs work by targeting the synthetic lethal interaction between BRCA1/2 and PARP. This review examines additional efforts to discover networks of synthetic lethal interactions and discusses both challenges and opportunities regarding the translation of new synthetic lethal interactions into the clinic.

Introduction: the emerging 'synthetic lethal' approach to cancer therapy.

Alterations to the tumor genome can be broadly classified into gain-of-function mutations in growth-enhancing genes (oncogenes) and loss-of-function mutations in growth-inhibitory tumor suppressor genes (TSG), as well as so called "passenger" mutations which can arise randomly as a result of impaired DNA repair but do not contribute to oncogenesis. Targeting oncogenes with either specific chemical inhibitors or therapeutic antibodies has proven to be highly effective for cancer therapy[1]. Indeed, the current practice of precision oncology has been built upon the success of these targeted agents[2]. Loss-of-function mutations in TSG have proven more difficult to target therapeutically, as it is not feasible with current technology to restore the function of mutated or deleted TSGs in the clinical setting[3]. However, rather than targeting a TSG directly, an emerging strategy is to identify 'synthetic lethal' genetic interactions between the TSG and other genes, such that simultaneous disruption of the function of both genes causes selective cell death (**Figure 1**)[4, 5]. Additionally, the process of genomic deletion of TSG often also deletes other genes with important functions; this 'collateral lethality' is another source of cancer-specific vulnerability[6, 7]. Given that as many as half of solid tumors may not harbor a known oncogene, or an oncogene such as *KRAS* that is not currently targeted by a specific inhibitor[8], the synthetic lethal approach has been proposed as a means to extend precision oncology to a significantly larger proportion of cancer patients[9].

The concept of synthetic lethality was first described in model organisms in the first half of the twentieth century[10, 11], however, it was only in 2014 that olaparib, the first drug to work via a synthetic lethal mechanism, gained regulatory approval for use in the clinic[12-15]. To date, the best-characterized synthetic lethal interactions are between *BRCA1* or *BRCA2* loss-of-function and inhibition of PARP1/2, both first reported in seminal papers in 2005[16, 17]. Mechanistically it is known that cells deficient for BRCA1 or BRCA2 have a reduced capacity for repairing double-stranded DNA breaks and are especially vulnerable to further perturbations in alternate DNA repair pathways[18]. This vulnerability can be exploited therapeutically by targeting components of single-strand break repair, such as PARP1/2, thus causing selective cell death in *BRCA1*$^{-/-}$ or *BRCA2*$^{-/-}$ cells[19]. It is now thought that the major mechanism underlying the lethality of PARP inhibitors is the trapping of PARP1 at sites of DNA damage, leading to stalled replication forks. Cells without the ability to perform Homologous Recombination (HR) are unable to repair these stalled replication forks, leading to lethal genomic rearrangements, and are thus especially vulnerable to PARP inhibitor mediated PARP1 trapping[20]. This model is supported by data showing that loss of PARP1 expression induces resistance to PARP inhibitors [21, 22]. Chemical inhibitors of PARP1/2 have now been in clinical trials for over 10 years, an experience that has validated the clinical utility of exploiting synthetic lethal interactions and led to the FDA approval of three drugs; olaparib, rucaparib, and niraparib[19, 23-25]. Given the clinical success of PARP inhibitors, there is now hope that the BRCA-PARP interaction is just the first of many synthetic lethal interactions that can be exploiting clinically[26].

Currently the application of the synthetic lethal approach to cancer therapy is limited by the relatively limited number of synthetic lethal interactions that have been discovered, as well as the fact that few interactions other than *BRCA-PARP* are well understood at a mechanistic level. Additionally, a growing number of studies have suggested that the majority of genetic

interactions are not absolute, but rather dependent on the cellular state as influenced by cell lineage, genetic aberrations, and environmental factors such as oxidative stress and the presence of chemotherapy[27-29]. In what follows, we examine how new technologies are being leveraged to identify novel synthetic lethal interactions, as well as current strategies to bring the synthetic lethal approach into the mainstream of cancer therapy.

How to define and measure synthetic lethal interactions

Genetic interactions are generally measured in terms of cell growth or viability, although it should be noted that it is possible to derive interaction measurements from other more complex phenotypes[30]. Terminology to describe genetic interaction dates back to the early 1900s, and has evolved over time as is described in prior reviews [5, 31]. In the context of the synthetic lethal approach to cancer therapy, the most commonly used terminology for genetic interaction comes from the high-throughput screens that have identified most of the known genetic interactions [27, 32-34]. Genes A and B are said to interact if the phenotype of the double knockout A,B ($P_{AB,observed}$) differs significantly from what would be expected based on the single knockout phenotypes of A and B individually ($P_{AB,expected}$). Genetic interaction is rare[5, 35, 36], so for most gene pairs there will not be a significant difference between $P_{AB,observed}$ and $P_{AB,expected}$. Quantitatively, a genetic interaction (εAB) between genes A and B in terms of phenotype P can be defined as:

$$\varepsilon AB = P_{AB,observed} - P_{AB,expected}$$

where $P_{AB,expected}$ is a function of the single knockout of A ($P_{A,observed}$) and the single knockout of B ($P_{B,observed}$)[33]. In cases where the measured phenotype, P, represents growth, if $P_{AB,observed}$ is less than $P_{AB,expected}$, the gene pair A,B is said to have a negative genetic interaction, also called a synthetic sick or in an extreme case a synthetic lethal interaction. If $P_{AB,observed}$ is greater than $P_{AB,expected}$, the gene pair A,B is said to have a positive genetic interaction, also called an epistatic interaction [5] B**Figure 2).** Quantitative genetic interaction scores are a continuous variable, to make a final determination of what gene pairs are synthetic sick or lethal, or

conversely epistatic, a threshold must be set. With regard to terminology other classifications of genetic interaction have been proposed[37], however these more complex systems require knowledge of directed relationships (A to B vs. B to A) which is not determined in most cases [38]. Large genetic interaction screens in both the budding yeast *S. cerevisiae* [32] as well as human cancer cells[39] have shown that $P_{AB,expected}$ can be reasonably approximated as the product of $P_{A,observed}$ and $P_{B,observed}$, simplifying the quantitative measurement of genetic interaction (note that in log transformed data this becomes the sum of $P_{A,observed}$ and $P_{B,observed}$). It is important to note that there are alternatives to the multiplicative model other for the determination of $P_{AB,expected}$, including additive, log, and minimum models, and that the choice of null model can have a strong influence on the results of screening data [34]. While the multiplicative model has been shown to best create the expected distribution of interaction scores close to zero for double knockout screens in yeast[40], it should be noted that the best model of $P_{AB,expected}$ may vary with different experimental methods. Multiple computational frameworks have been developed to robustly score genetic interactions from experimental data from either human or model organism screens[27, 37, 39, 41, 42]. However, given that calculation of genetic interaction requires precise measurement of single gene knockout fitness, a method that incorporates serial measurement over a time-course has recently been developed to improve the precision of fitness single gene measurements and thus the power to detect genetic interactions[27].

In addition to the above framework for measuring synthetic sick or lethal interactions from loss-of-function perturbations, it is also possible to quantitate interactions with gain-of-function perturbations. The term synthetic dosage lethality is used to describe a genetic interaction where a gain-of-function in gene A is lethal when combined with loss-of-function of gene B[43-45]. In cancer, synthetic dosage lethal interactions are an additional means to target tumors with gain-of-function mutations, especially in cases with oncogenes such as KRAS that are not

directly druggable (**Figure 1**) [46]. High-throughput screens for synthetic dosage lethal interactions have been performed in yeast[44, 47] as well as human cancer cells[43, 48, 49].

General mechanisms of synthetic lethal interactions

Quantitative genetic interactions scores can be used to construct pathway connections between genes, as negative and positive interactions are associated with different pathway relationships. However, there are several different pathway relationships that will give rise to synthetic lethal (negative) interactions, as is illustrated in **Figure 2**. If two genes in unrelated pathways are both knocked out, the result is a neutral interaction, (interaction score very near zero as there is no difference between observed and expected double knockout fitness). The classic case of synthetic lethal interaction is the knockout of two genes in separate, but parallel pathways. Synthetic lethal interactions can also arise from genes within the same pathway with the accumulation of multiple partial pathway defects, if there is within-pathway redundancy, or redundancy within a protein complex[50]. Mutations in reversible pathways can result in synthetic lethal interactions if the combined loss-of-function results in the buildup of a toxic intermediate. Finally, “capacitor” proteins such as chromatin regulators and heat shock proteins, which buffer perturbation to many genes, have many synthetic lethal interactions[51]. Positive, or epistatic, interactions generally occur when two genes are in the same pathway, when the inactivation of a single gene is sufficient to inactivate the entire pathway. When applying these concepts to cancer biology it is important also to consider if a mutation is expected to result in complete or partial loss-of-function. While homozygous deletion or frameshift mutations would be expected to result in complete loss-of-function, most point mutations in TSG result in partial, rather than complete inactivation. With only partial loss-of-function, mutations within the same linear pathway will produce synthetic lethal interactions (**Figure 2A**).

How to discover therapeutically relevant synthetic lethal interactions

Given the clinical success of PARP inhibitors, there is a growing effort to identify more synthetic lethal interactions relevant to cancer therapy. Current efforts to map synthetic-lethal interactions can be separated into four basic categories. First are statistical approaches that leverage large populations of tumor genomes. These analyses are based on the assumption that if genes A and B are synthetic lethal, tumors with simultaneous loss-of-function of both A and B should have reduced fitness, thereby creating evolutionary pressure to prevent the co-mutation of the synthetic lethal pair. Statistically this is detected by performing mutual exclusivity analysis to test if there is a significant depletion of co-mutated tumors[52, 53]. This approach has the advantage of directly examining patient populations; however, its statistical power is limited by the frequency of TSG mutation. For example, the expected co-mutation rate of two genes with 2% mutation frequency would be 0.04%, meaning only one sample in 2,500 tumors would be expected to be co-mutated. Thus tens of thousands of tumors would need to be sequenced to identify a statistically significant depletion of co-mutation. Given the so called 'long-tail' of cancer mutations, meaning the majority of genes are mutated in far fewer than 1% of tumors[54], even with 10,000 samples fewer than 5% of all gene pairs could be called as significantly mutually exclusive[9]. To increase statistical power, some approaches have combined copy number alterations (CNA) and gene expression with somatic mutation[55, 56]; however, one concern with using CNA data in this manner is that deletions and amplifications frequently span many genes, making genes in the same chromosomal region appear to be co-mutated.

In addition to statistical concerns, another concern for using mutually exclusive gene mutations to infer synthetic lethal interactions is that such effects can occur for reasons other than synthetic lethality[57]. For example if mutation in gene A is sufficient to provide a proliferative advantage, and further mutation of gene B does not provide additional growth advantage, the pair A,B would be mutually exclusive but not necessarily synthetic lethal. Such a situation could occur if genes A and B were associated with different intrinsic tumor subtypes

that had unique driver genes, such as *TP53* mutation and *TERT* promoter mutation in low grade glioma[58].

A second approach leverages evolutionary conservation and the fact that it is possible to conduct ultra-high throughput genetic interaction screening in model organisms such as *S. cerevisiae*. Many important human TSGs, including the DNA mismatch repair genes *MSH2* and *MLH1,* were first identified and studied in yeast[59-64], and yeast has also proven to be an experimentally tractable model system for mechanistic study of DNA repair and the DNA damage response, which are highly conserved eukaryotic functions[65, 66]. Initially proposed over 20 years ago[67], this cross-species approach was enabled by the creation of a knockout library including essentially every gene in *S. cerevisiae* [68, 69], technological advances for the high-throughput mating of yeast[33, 36, 70, 71], and the development of quantitative scoring methods[37, 41, 42]. Such methods allow for genetic interactions to be measured in an unbiased manner and at very large scale, with minimal off-target effects since the genes are disrupted by complete and specific knockout of the open reading frame. Additionally this screening technology has been extended to the fission yeast *S. pombe* [72], allowing for comparative analysis of these divergent species [73-75]. With regard to identifying clinically relevant synthetic lethal interactions, the model organism approach is inherently limited to testing genes that are evolutionarily conserved, however numerous such interactions have been observed [73, 75, 76].

A systematic study to determine the degree to which synthetic lethal interactions were conserved between *S. cerevisiae* and human cancer cells found that observing a synthetic lethal interaction in yeast increased the likelihood of a synthetic lethal interaction between the orthologous human gene pair by approximately four fold[9]. However, if the yeast interaction was observed in multiple environmental contexts this ratio increased to ten-fold, and for gene pairs annotated in the same biological process the likelihood of human interaction increased to ~20-fold from baseline. Of note, Costanzo et al. have published a comprehensive interaction

map of nearly all pairwise combinations of the roughly 6,000 *S. cerevisiae* genes, identifying 550,000 synthetic lethal and 350,000 epistatic interactions from approximately 23 million double mutant combinations [32], and this work has recently been expanded to include trigenic interactions[77]. These systematic efforts to profile genetic interactions in *S. cerevisiae*, as well as related efforts in other model organisms[78], have led to the observation that genetic interactions are significantly more likely to occur between genes in different pathways relative to genes in the same pathway[79] (**Figure 2**). This "between-pathway" observation has been leveraged to develop a predictive algorithm able to identify interactions contributing to risk of breast cancer from Genome-Wide Association Studies (GWAS) [80]. Similarly it has been observed in both model organisms and human cancer cells that genes with large individual fitness effects are enriched for synthetic lethal interactions[9, 27]. Thus in addition to identifying specific gene-gene interactions, high-throughput genetic interaction studies in model organisms can aid the discovery of human synthetic lethal interactions by identifying general principles of how network topology influences genetic interaction [29]. Similarly screening in model organisms has identified that genes involved in chromatin regulation are enriched for genetic interaction[32, 81].

A third approach involves direct experimentation in human cancer cell lines using either high-throughput functional genomic or chemical screening. These experiments are often performed in isogenic cell line pairs in which a cancer cell line is engineered to carry a mutation in a common cancer gene such as *TP53* [82]or *KRAS* [83]. These cell lines, which at the DNA level differ only in the mutational status of a single gene, are then screened against either a chemical library or a single gene knockdown/knockout functional genomic library with the goal of finding perturbations selectively lethal to the mutated cell line. Often the isogenic pair is labeled with fluorescent markers allowing for competitive growth in the same experimental well, which has been shown to reduce technical variability[84]. Screening with small molecule probes is generally performed with robotic automation in arrayed microtiter plates (96, 384, or 1536 wells

per plate), and has the advantage that the identified molecules are already lead compounds for medicinal chemistry efforts[26]. However, in high-throughput experiments it is not feasible to optimize the screening dose of each compound, and it can be difficult to identify the relevant target of compounds of interest. For functional genomic screening, small interfering RNA (siRNA) transfected into cells in arrayed, mircrotiter plate format was the initial technology of choice[26, 85] however the scale of these experiments was increased by using short hairpin RNA (shRNA) which could be barcoded, packaged into lentivirus, and screened in pooled format[86]. Despite the initial success and enthusiasm behind RNAi screens, given issues with off target effects and reproducibility between screens RNAi screening most high-throughput functional screens are now performed with CRISPR technology[28, 87, 88]. In the CRISPR-Cas9 system a guide-RNA (gRNA) forms a complex with the nuclease Cas9, targeting genomic sequences homologous to the gRNA and allowing Cas9 to induce a double strand break (DSB)[89-95]. Compared to RNAi screens, CRISPR-Cas9 screens have shown superior performance in identifying essential genes, particularly lowly expressed genes[96]. Additionally, transcriptional promoters or repressors can be attached to catalytically inactive Cas9 to perform activation (CRISPRa) or inhibition (CRISPRi) screens[97].

The decreased cost and increased throughput afforded by the pooled format has allowed for a move away from isogenic cell line pairs to large populations of cancer cell lines[98, 99]. The largest such effort, Project Achilles, now contains RNAi data for over 500 cell lines and CRISPR-Cas9 data for over 400 cell lines[100]. By aggregating the genomic characterization of the cell lines with the functional knockout or knockdown data, lineage and mutation specific dependencies, including synthetic lethal interactions, can be determined[101]. Screening a large population of cancer cell lines avoids known issues with isogenic pair cell lines, namely the artificial context of the induced mutation and genetic drift between the pair[26], and it also has the advantage of allowing for consideration of cell lineage in addition to mutation status. Similar to isogenic pair screens where both loss-of-function and gain-of-function mutations can be

modeled, screens performed in large numbers of cell lines can detect both synthetic sick or lethal interactions and synthetic dosage lethal interactions[49]. Large data sets such as Project Achilles have allowed for the identification of, and bioinformatic compensation for, off-target effects for both RNAi and CRISPR screens[102, 103], and methods to integrate single knockout data from different screens have been developed to further aggregate these data[104]. However, identifying synthetic lethal interactions from screening large numbers of cell lines is ultimately a population-based approach, and as such faces similar challenges including bias towards the most commonly mutated genes.

A fourth approach to identify synthetic lethal interactions is to perform combinatorial, rather than single, gene disruptions in human cell lines. This approach is most similar to the double perturbation screens that have been run in model organisms for many years (see above), but it is only recently able to be performed in high-throughput. Early human double perturbation screens used either pairwise siRNA knockdowns[39, 105] or combinations of siRNA and drug treatments[106]; however, these were limited in scale. With the shift to CRISPR-Cas9 technology, multiplex targeting for the knockout of two genes simultaneously is enabled by the delivery of multiple gRNAs to a cell[107]. Generating both loss-of-function mutations experimentally avoids the statistical issues associated with population-based screens and allows for infrequently mutated genes to be assayed without bias. With these advances in CRISPR technology, it is now possible for the first time to systematically map genetic interaction networks in human cancer cells. Indeed, multiple such interaction maps have already been generated[27, 108-110] with many more under way[111]. Additionally, orthologous CRISPR proteins for different bacterial species (*Staphylococcus aureus* as opposed to standard *Streptococcus pyogenes*) are being developed to avoid Cas9 binding site competition[112] and have also been used to activate one gene while inhibiting the second[38].

Challenges to the implementation of the synthetic lethal approach in cancer therapy

Although high-throughput screening efforts to identify more synthetic lethal interactions remain an important component in furthering the synthetic lethal approach to cancer therapy, it should be noted that the number of literature reported synthetic lethal interactions is now in the thousands[113]. Although the majority of those interactions have not been extensively validated, that number is a sharp contrast to the number of synthetic lethal interactions that can currently be exploited in the clinic, which remains at one. What therefore are some of the most important reasons for this bottleneck?

Perhaps the greatest barrier to the development of synthetic lethal therapies is the molecular heterogeneity of cancer. It is now well known after the sequencing over 10,000 tumors in The Cancer Genome Atlas (TCGA) that cancer is tremendously diverse at the molecular level[114]. Even two tumors that are similar in terms of both anatomic origin and histologic appearance have little overlap in terms of somatic mutations; this heterogeneity even exists from cell-to-cell with a single patient[115]. It is clear from many anecdotal examples, such as the success of single agent BRAF inhibition in *BRAF* V600E melanoma, but subsequent failure in *BRAF* V600E colon cancer, that chemo-genetic relationships are not absolute, but rather dependent on factors including cell lineage and the presence of other genomic aberrations[116]. The results of early genetic interaction mapping experiments in a small number of cell lines suggest that synthetic lethal interactions are much more context dependent than single gene fitness effects, with only ~10% being conserved across multiple cell lines[27]. Given the known heterogeneity of cancer it will be important to identify those synthetic lethal interactions that are conserved in the greatest number of contexts (cell lineage, presence of other mutations, presence when cell is treated with chemotherapy) for prioritization for therapeutic development. This will require extensive validation of each potential synthetic lethal hit in those multiple contexts. Furthermore, a mechanistic understanding of what drives the synthetic lethal relationship will also be critical as even strong interactions, like BRCA-PARP, are not conserved universally, such that mechanistic understanding can help predict when

synthetic lethality will be lost. For instance when cells have *53BP1* loss-of-function in addition to *BRCA1* loss-of-function, homologous recombination (HR) is not suppressed and the synthetic lethal effect with PARP inhibition is lost[117].

To achieve truly precision oncology a better understanding of how the molecular heterogeneity of cancer impacts the context specificity of a given therapeutic intervention will be required. In this regard it will be just as important to understand specific cases when a drug will not work as it is to know instances where it will be effective. The likelihood that most synthetic lethal interactions will be context specific is not necessarily a barrier to clinical translation, as long as the context specificity is known in advance; patients with a molecular profile suggesting lack of response will be excluded from getting that particular drug. The screening of colorectal tumors for *KRAS* and *NRAS* mutations, which predict lack of response to anti-EGFR antibodies, is an example of how knowledge of context specificity is already being applied in clinical practice[118]. Unlike the example of *KRAS* mutation in CRC, however, which was discovered from a retrospective analysis of patients already treated with anti-EGFR antibodies, moving forward it will be critical to develop knowledge of context dependence in preclinical models.

In addition to these issues of context specificity, the identification and validation of drug targets from *in vitro* screening data has also been challenging. Whereas oncogenic driver genes are easily identified based on recurrent mutation patterns[119] and can be easily validated as functionally important, the identification of potential synthetic lethal targets requires a combination of high-throughput functional genomic screening and bioinformatic analysis. This was a particular challenge when RNA interference was the primary technology used for screening in human cancer cells due do frequent off-target effects, resulting in false positive interactions[120]. However, advances in functional genomic technology, most notably the development of the CRISPR-Cas9 system[89], has largely corrected these issues with specificity and led to identification of many promising synthetic lethal targets. These technological advances are driving increased discovery efforts both in academia and industry,

with at least four new biotech firms focused on identifying druggable synthetic lethal interactions launched since 2016[121].

Cancer as a network-based disease

Just as ignoring context specificity will lead to treatment of non-responsive tumors, limiting the concept of synthetic lethal interaction to just a single gene pair, such as *BRCA1* and *PARP1*, will exclude many responsive tumors. It has been widely discussed that cancer is a disease that arises because of the action of hallmark cancer pathways[122, 123]. Although any particular mutation or mutated gene may be a rare event when viewed independently, the key hypothesis of the hallmark pathway model is that these rare events will be found to converge on a smaller number of protein complexes, signaling cascades, or transcriptional regulatory circuits. This model is supported by multiple lines of evidence including mutual exclusivity of multiple mutations in the same hallmark pathway[57]. Thus, the key question is not whether cancer is a pathway or network-based disease – this hypothesis is familiar and increasingly well supported – but how best to apply this principle to the analysis and interpretation of cancer genomes.

A highly related, central problem in cancer genetics is to understand how different variations in DNA sequence, dispersed across a multitude of genes, can nonetheless elicit similar phenotypes and patient outcomes. A better understanding of this complex genotype-to-phenotype relationship will aid greatly in predicting what chemical interventions are most likely to kill a cancer cell[111]. To cope with genotypic heterogeneity, in recent years an increasing number of studies have shown that different genetic drivers of a trait can be recognized by their aggregation in molecular networks[124-126]. Rather than associating genotype with phenotype directly, variations or mutations in genotype are first mapped onto knowledge of molecular networks; affected sub-networks are then associated with phenotype.

Given this network architecture of cancer cells it is expected that synthetic lethal interactions will converge into protein-protein and genetic interaction networks. The idea of network convergence is that an interaction between two single genes, A and B, can generalize to include genes from the same pathway or complex of either gene, thus the synthetic lethal relationship is between two cellular functions or pathways rather than two genes[79]. One example of this network concept of synthetic lethality is termed “BRCAness,” which describes tumors without *BRCA1/2* mutation that display defects in homologous recombination, presumably due to mutations in other related DNA repair genes such as those in the Fanconi Anemia pathway[127]. Although the best way to identify the BRCAness phenotype is not yet determined, already there is clinical evidence showing that BRCAness can be used as a prognostic and predictive biomarker[24, 128-130]. An important caveat is that networks of synthetic lethal interactions will still be context dependent; this is seen in the BRCAness example where response to olaparib was significantly less in *BRCA1/2* wild-type breast cancer relative to ovarian cancer[128, 131].

The future of the synthetic lethal approach to cancer therapy

Given the growing basic research investment in the identification, validation, and mechanistic characterization of cancer-relevant synthetic lethal interactions, one might expect that the number of approved drugs that work in a synthetic lethal fashion will continue to grow. As technological advances continue to expand capabilities for genetic interaction mapping in human cancer cells, more will be learned about the context specificity of these interactions. One context that will be particularly important to consider is the influence of the addition of other targeted or traditional chemotherapeutic drugs[132], as understanding these complex interactions could allow for the development of successful drug combinations. Given that response to a single targeted agent in a solid tumor is transitory and essentially always leads to drug resistance identify effective drug combinations to target resistance pathways will be

critical[133]. Because the number of new cancer therapies is growing rapidly it will be critical to predict synergistic combinations in the preclinical setting as there will simply not be enough clinical trial patients to test all combinations. With regard to clinical testing it will be important to identify dosing regimens, potentially cycling different synthetic lethal partners, to avoid overlapping toxicity from multi-drug cocktails. Recognizing that tumors will almost certainly evolve resistance to effective synthetic lethal therapies, it will be important to design trials to allow for serial biopsy, perhaps via circulating tumor DNA, to both identify resistance clones and confirm the on-target effect of the therapy.

If publications on synthetic lethality continue to grow at current rates by 2020 there will be over 300 publications a year and over 500 publications a year by 2025 (**Figure 3**). Such a volume of information will overwhelm the ability of any individual practitioner to review and comprehend without the assistance of bioinformatic decision support tools. An important first task will be organizing these network data in a common database using standardized terminology and in a machine readable and searchable format; the Network Data Exchange (NDEx) is one platform that has been created for this purpose[134]. Given the sheer volume of data, understanding the complexities of the context dependence of synthetic lethal interactions and other tumor specific vulnerabilities such that effective therapies can be predicted from a tumor's molecular profile will likely require the incorporation of machine learning techniques. In particular, deep learning models, patterned after the neural network of the human brain[135], have shown initial successes in various biological applications such as drug discovery[136], DNA/RNA protein binding and interpretation of noncoding variants[137, 138]. Recognizing the importance of understanding the mechanism by which input data are connected to outputs, recently machine learning models that are both predictive and descriptive of biological systems have been designed to replace prior "black box" methods[139] (**Figure 4**).

While these approaches have been shown to be robust in model organisms, currently not enough of the human genetic interaction space has been mapped to allow for similar in human cancer cells. However, moving forward organizing the expanding experimental data supporting cancer-specific vulnerabilities, the context specificity of those vulnerabilities, and a wealth of prior knowledge about genetic and protein interaction into a unified network framework should allow for the training of such a descriptive artificial intelligence (AI) algorithm. In addition to machine learning methods, these same experimental training data could be used to build either logic models or quantitative models of synthetic lethality[140]. These methods have already been used with some success to predict effective drug combinations[141], and will clearly improve as more high quality data become available. Thus it is anticipated that in the future once a tumor is biopsied and profiled for gene expression and somatic mutations that these data will be fed into a predictive algorithm, trained using a descriptive AI method that leverages prior knowledge of physical and genetic interactions of the cancer as well as logic models built from these data. This unified predictive algorithm will then evaluate networks of synthetic lethal interactions to identify the drugs most likely to be effective for a specific patient as well as the mechanisms underlying why the tumor will be sensitive to that drug or drug combination.

Conclusions

Because loss-of-function mutations and gene deletions are common events in cancer, targeting these via synthetic lethal interactions has great promise to extend precision oncology to tumors without dominant oncogenic drivers. However, although the clinical success of PARP inhibitors has validated the synthetic lethal approach to cancer therapy, currently the impact of synthetic lethality is currently limited by the small number of synthetic lethal interactions that are understood mechanistically. New screening methodologies are currently aiding the discovery of novel synthetic lethal interactions and providing information about the context-specificity of

known interactions. Understanding context-specificity will be a critical element in selecting which patients are most likely to benefit from a given therapy. However, as these data continue to grow, descriptive and predictive AI methods will be needed to assist clinical decision making to determine how best to select which tumors will respond to a drug or drug combination.

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Figures:

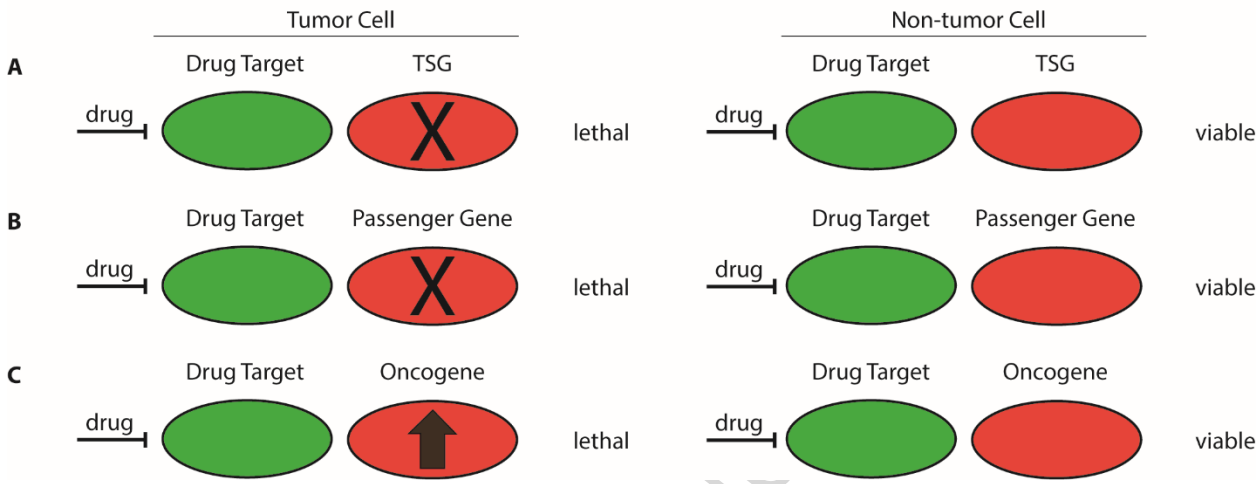

Figure 1. Classes of tumor specific vulnerabilities. Synthetic lethal interaction **(A)** involve a loss-of-function mutation or deletion in a tumor suppressor gene (TSG); when the partner gene is inhibited with a drug the function of both genes is now lost in the tumor cell with lethal effect. Non-tumor cells, which retain function of the TSG, remain viable. Conditional lethal interactions **(B)** are similar to synthetic lethal interactions but involve loss-of-function mutations or deletions of passenger genes. Gain-of-function mutations or overexpression of oncogenes can also create tumor specific vulnerabilities; this is called synthetic dosage lethality **(C).**

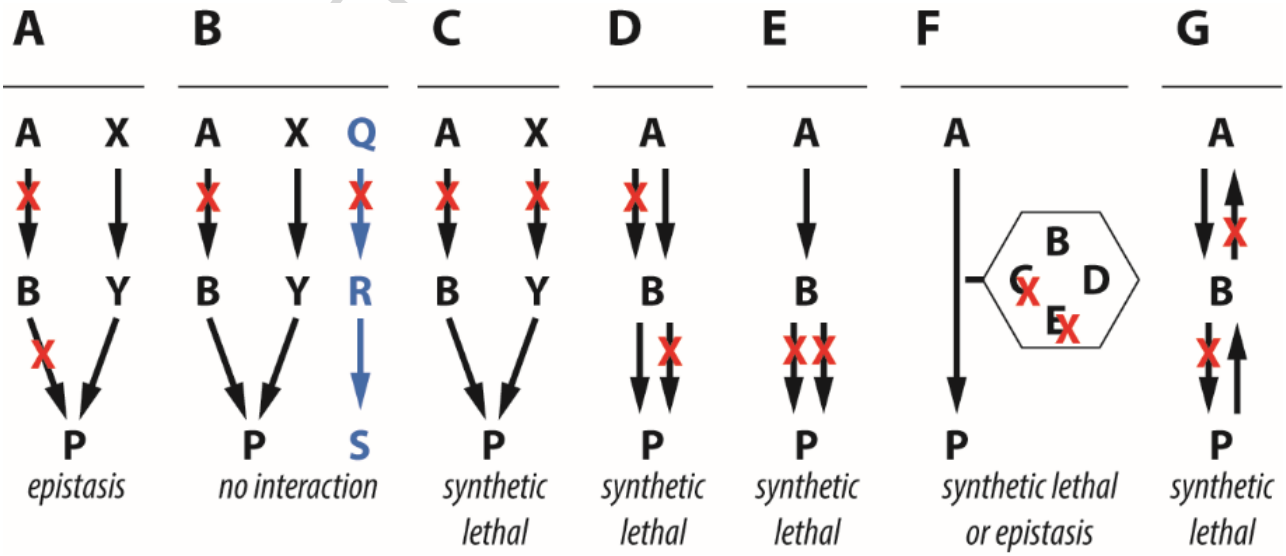

Figure 2. Classes of genetic interactions. In these diagrams a range of substrates (letters) are converted by a series of proteins (arrows) by various different pathways to create product P which is essential for cell viability. Red X's indicate protein function is completely lost due to mutation (or deletion). When two genes from the same pathway are mutated the result is a positive or epistatic interaction **(A)**, however note that if these mutations only caused partial loss-of-function the result would instead be a synthetic lethal interaction. When two genes in unrelated pathways are mutated there is no interaction (also called neutral interaction) **(B).** Mutation of two genes in parallel pathways leading to an essential product will result in cell death, this is the classic case of a synthetic lethal **(C).** Synthetic lethal interactions can also arise from genes within the same pathway when there is accumulation of multiple partial pathway defects **(D)**, or within pathway redundancy **(E)**. Mutations within the same protein complex could produce a synthetic lethal interaction if loss of a single protein completely inactivates the complex, or an epistatic interaction if the first mutation results in only partial inactivation of the complex. Synthetic lethal interactions can also occur when mutations in reversible pathways lead to the accumulation of a toxic intermediate (B in this diagram) **(F)**.

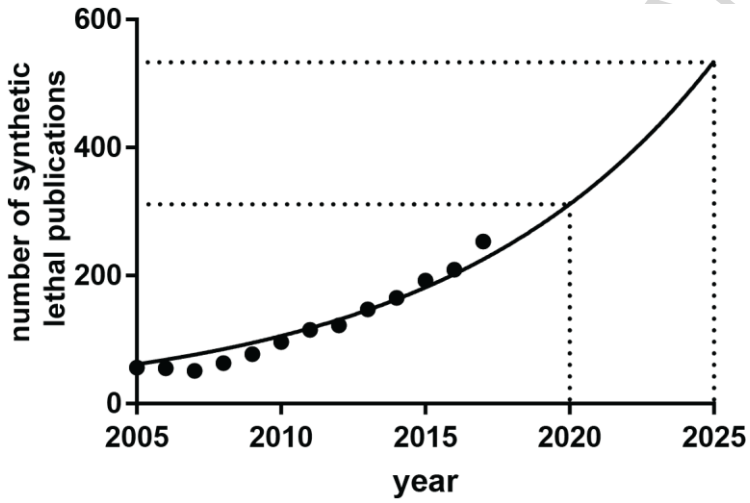

Figure 3. Number of synthetic lethal publications per year.

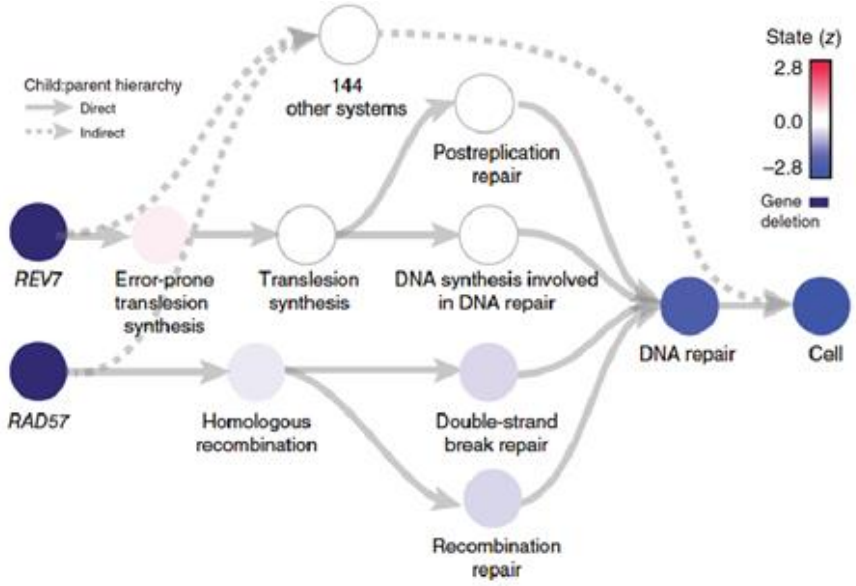

Figure 4. Example of a machine learning algorithm that provides knowledge about what cellular systems are being altered to explain the synthetic lethal relationship between REV7 and RAD57.

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