Opinion:

Synthetic lethality and cancer – penetrance as the major barrier

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Running title: synthetic lethality in cancer

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Abstract

Synthetic lethality has long been proposed as an approach for targeting genetic defects in tumours. Despite a decade of screening efforts, relatively few robust synthetic lethal targets have been identified. Improved genetic perturbation techniques, especially those based on CRISPR-Cas9 gene editing, have resulted in renewed enthusiasm for searching for synthetic lethal effects that operate in cancer [1]. An implicit assumption behind this enthusiasm is that the lack of reproducible targets identified can be attributed to the limitations of RNA interference technologies. Here, we argue that a bigger hurdle is that most synthetic lethal interactions are not highly penetrant, i.e. are not robust in the face of the extensive inter- and intra- molecular heterogeneity seen in tumours. We outline strategies for identifying and prioritising those synthetic lethal interactions most likely to be highly penetrant.

Highlights (900 characters, including spaces to be submitted as separate word doc)

- The development of CRISPR-Cas9 technologies has caused renewed enthusiasm for identifying synthetic lethal effects that operate in cancer
- Whilst some of this enthusiasm is warranted, we highlight the one factor limits the utility of synthetic lethal interactions as cancer treatments – most synthetic lethal effects are private to individual model systems and display incomplete penetrance
- Experimental and computational methods for defining synthetic lethal penetrance are discussed
- We predict the biological principles that might determine the extent of synthetic lethal penetrance

The search for synthetic lethality in cancer

Searching for “synthetic lethality” and “cancer” in Pubmed will reveal a plethora of review articles that detail how a genetic phenomenon first observed in model organisms has led to the development of novel therapeutic approaches in cancer. ‘Synthetic lethality’ (see Glossary) was initially used to describe an incompatibility between pairs of alleles in fruit flies, but has broadly come to indicate any instance where perturbation of two genes individually is well tolerated but in combination results in cell death. The first clinical application of this concept led to the regulatory approval of a drug class, known as PARP inhibitors, for the treatment of breast or ovarian cancers with mutations in the *BRCA1* or *BRCA2* tumour suppressor genes [2]. The potential of exploiting synthetic lethality to target tumours has been such that many papers have been published identifying synthetic lethal effects associated with individual cancer ‘driver’ genes. These ‘gene centric’ efforts have been complemented by near industrial scale ‘unbiased’ efforts to map the genetic dependencies of large panels of tumour cell lines [3-6]. These studies provide an overview of the genes necessary for the growth of individual cell lines and, in combination with genotype information, can be

used to identify synthetic lethal effects. The ultimate goal of these efforts is to identify vulnerabilities in human cancers that can be exploited using drugs.

Mapping out the various synthetic lethal effects that operate in human cancer is clearly a worthwhile pursuit. Despite some major advances in developing treatments that target specific vulnerabilities in cancers, the vast majority of patients are still treated with approaches that could be grouped into one of three categories; remove the tumour (surgery), poison it slightly more than normal tissue is harmed (chemotherapy) or irradiate the tumour (radiotherapy), hoping that any deleterious effect on normal tissue is limited. The relative paucity of targeted treatment approaches (i.e. those that are based on an understanding of the molecular biology of the individual's disease) is such that new targets are sorely needed. There was much hope that the exploitation of synthetic lethality would deliver some of these targets [7, 8]. Of course, the gap between hope and reality has been somewhat greater than many imagined - but why? Why, after a decade or so of concerted effort aimed at developing synthetic lethal treatments for cancer, have we only a handful of successful examples?

Much of the blame has been attributed to technical problems – especially those associated with RNA interference (RNAi), until very recently the primary workhorse technology used to identify synthetic lethal effects. RNAi approaches are limited by “off-target” effects, where the RNAi reagent inhibits additional genes to the target gene of interest (GoI), as well as silencing efficiency issues, where the RNAi reagent does not appear to inhibit the GoI to any great extent. Both of these issues have almost certainly led to a series of false positives and false negatives in synthetic lethal experiments [9]. Much enthusiasm has thus been attributed to newer technologies that exploit CRISPR-Cas9 gene editing, where targeting efficiency is often greater and where off-target effects have somewhat less impact [1].

However, we would argue that the technical aspects of approaches such as RNAi, whilst somewhat limiting, are not necessarily the greatest factor that has limited the search for SL effects with clinical utility. Perhaps of greater

importance is the issue of penetrance, described below, which will remain a key issue, even with the application of CRISPR based approaches.

The problem with synthetic lethality: incomplete penetrance

In 2011, we and others highlighted that one factor limiting the utility of synthetic lethal interactions as cancer treatments is the extent to which these effects are dependent upon the genetic background in which they are measured [10]. We proposed that the synthetic lethal effects that are readily abrogated by changes in additional genes (***soft synthetic lethal effects*** **Figure 1**) would not be as ideal as cancer therapy targets when compared to synthetic lethal interactions that are relatively resilient to additional molecular changes (***hard synthetic lethal effects*** [10]) (**Figure 1C**). These concepts of hard and soft synthetic lethality are really an extension of the concept of ***penetrance***. When used in population genetics, penetrance describes the fraction of individuals carrying a particular genetic marker who also exhibit an associated phenotype. When applied to synthetic lethality in cancer, we propose the term ***synthetic lethal penetrance (SLP,*** see Glossary) be used to describe the fraction of tumour cell clones with a specific genetic alteration (e.g. a cancer driver gene mutation) that undergo cell death when a synthetic lethal target is inhibited. Ideally, synthetic lethal treatments should have ***complete penetrance*** (**Figure 1D)** with the presence of the driver gene and inhibition of its synthetic lethal partner always leading to tumour cell death, regardless of the rest of the molecular composition of the tumour cell (the reverse of this scenario is ***incomplete penetrance***). What is perhaps more realistic is that we only select highly penetrant effects for clinical assessment and disregard SL effects with low penetrance before these reach the clinic.

Despite the importance of assessing the penetrance of synthetic lethal interactions, it is clear that this issue has received far less attention than improvements in gene perturbation approaches. Even in model organisms such as budding yeast, where large scale synthetic lethal screens have been performed for over a decade, most screens are performed in a single defined

genetic background. This means that we have relatively little idea of how dependent synthetic lethal effects are upon their genetic background, or how penetrant they might be across different backgrounds [11].

In cancer, the oncogene *KRAS* is perhaps the gene most widely screened for SL interactions (reviewed in [12]). Despite the large number of screens performed, there are very few examples of KRAS SLs that replicate across multiple, distinct, studies [12, 13]. As most screens have been performed using RNAi based approaches, much of the lack of reproducibility has been attributed to the limitations of this technology and to variations in the experimental approaches used [9, 13]. However, a recent study suggests this might not be the key issue. Elledge and colleagues performed genome-wide CRISPR synthetic lethal screens in two pairs of colorectal tumour cell lines to identify KRAS SLs [14]; despite the use of CRISPR and the similarity of the experimental approach, most SLs identified were private to a single cell line. This suggests that neither the limitations of RNAi nor differences in screening approaches are sufficient to explain the variation in identified SLs.

Recently two groups have developed pipelines to identify genetic interactions through combinatorial CRISPR screening (i.e. inhibiting two genes simultaneously) and applied these approaches to the analysis of multiple tumour cell lines (TCLs) [15, 16]. In both cases, many observed SLs were cell-line specific, with Shen and colleagues noting that only ~10% of the SLIs identified were observed in more than one TCL [15]. To get a more accurate picture of the cell-type specificity of SLs, statistical approaches that take into account experimental reproducibility are required, similar to those developed to estimate condition specific genetic interactions in yeast [17]. Nevertheless, it seems that many SLs are TCL specific.

Consistent with these preliminary observations in TCLs are results in model organisms, where it has been shown that many pairwise genetic interactions (including SLs) are modified by the deletion of additional genes [18-20] and many genetic interactions are only observed in a specific genetic background [21, 22].

Reasons for incomplete penetrance (Figure 1E)

Cell type specific networks. The extensive variation in the gene-regulatory and signalling networks that are active in different cell types is a likely explanation for some of the tissue specificity of targeted agents in cancer. A well-established example involves addiction to the *BRAF* oncogene - melanoma cells with activating mutations of *BRAF* are highly sensitive to its inhibition but colorectal cancer cells with the same mutation are relatively insensitive [23]. This has been attributed to differential wiring of the signalling networks in the two cancer types. Such differences in cell-type specific networks likely impact synthetic lethal interactions also. A recent screen identified a SL between PREX1 and RAS in a panel of acute myeloid leukemia (AML) cell lines but not in other cell types [24]. The authors attributed this to differential expression of PREX1 and its paralogs in different cell types - AML cell lines express PREX1 but not its paralog TIAM1, which was expressed in other cell types. Inducing expression of TIAM1 in AML cell lines partially rescued the RAS/PREX1 SLI suggesting differential expression patterns may explain cell-type specific SLs.

Genetic and epigenetic heterogeneity. Even amongst cells from ostensibly the same tissue type there can be major genetic and epigenetic heterogeneity within and between tumours, which may also account for the variation in synthetic lethal effects observed in different tumour cells. A pair of tumour cells that share an alteration in a common driver gene may differ in the status of many other genes and some SLIs might be abrogated by these additional alterations. For example, a recent study identified a SLI between *XPO1* and *KRAS* in many lung cancer cell lines, an effect that was reversed by alteration of *FSTL5*, another gene frequently altered in lung adenocarcinomas [25]. Such 'synthetic rescue' effects can also be identified in the context of acquired resistance to synthetic lethal treatments, e.g. loss of 53BP1 can result in resistance of *BRCA1* mutated tumours to PARP inhibitors [26]. In contrast to the situation where alteration of a specific gene may result in resistance to an SL, some SLIs may only be evident in cells with very specific combinations of

mutations, e.g. lung cancers cell lines with KRAS mutation and loss-of-function alterations of KEAP1 are sensitive to inhibition of glutaminase [27].

Experimental approaches to identify highly-penetrant synthetic lethal interactions

Several approaches exist for empirically defining the penetrance of SL effects. Most SL screens are initially carried out in single isogenic model systems (**Figure 3A)**, which, when used in isolation, merely indicate that the identified effects operate in one particular genetic background. They do not provide any indication of the penetrance of the effects. An alternative approach is to perform screens in a large number of non-isogenic models, classified according to the status of the gene of interest (GoI) (**Figure 3B**), and identify those genes whose inhibition selectively inhibits the growth of cell lines with alterations in the GoI [13]. The advantage of this approach is that the molecular heterogeneity present across the cell line panel used can provide: (i) a rigorous test of whether a SL effect is highly penetrant; and (ii) an assessment of the SL phenotype in the setting of different co-occurring driver mutations (e.g. *KRAS* & *APC*, *BRCA2* & *TP53*) that might not be found in single model screens.

However, performing and interpreting large-scale non-isogenic screens comes at some cost, in both economic and logistic terms. Moreover, the associations identified between GoI and SL partners are often artefacts caused by some other, often unknown, molecular feature that segregates with the driver gene mutation, making a causal association between the GoI and the identified effects difficult to establish. This issue could be resolved by the use of isogenic screens alongside non-isogenic systems. Alternatively, penetrance could be assessed by the use of multiple different isogenic systems for the GoI, with each isogenic cell pair being derived from a different cell lineage (**Figure 3C**). This could prove powerful, assuming there is a requisite level of molecular heterogeneity between the parental cell lines used. To ensure an extreme degree of genetic heterogeneity, such approaches could also incorporate isogenic screens from distinct species

(e.g. mouse-derived cell). An SL effect that is conserved between distinct species is by definition, resilient to the large-scale molecular re-wiring that accompanies speciation Finally, one additional method to assessing the penetrance of an SL effect might be to carry out synthetic rescue screens in isogenic model systems to see how many different molecular mechanisms there are of abrogating an SL (**Figure 3D**).

The idea of exploiting model organisms to identify and validate human SL interactions is not new [8] and is still commonly used. Recently, a number of groups have successfully mined SL interactions from yeast, which encompasses the most comprehensive delineation of SL interactions, to identify candidate therapeutic targets in human cancer [28-33]. However, the reverse situation, where model organisms are used to assess the resilience of a SL interaction identified in human cells, to molecular change, seems an approach far less used. There are of course significant issues to consider when using a comparative approach. The most obvious is whether model organisms have clear orthologs of the human GoI and/or whether molecular processes that are specific to human cells exist in model organisms. For example, it is difficult to imagine the relevance of modelling metazoan specific signalling pathways or cell-cell communication networks, in non-metazoan, unicellular yeast. In this case, the use of other metazoans (e.g. *C. elegans* or *Drosophila melanogaster*), might be more appropriate [34-36].

Understanding the extent to which SL interactions are conserved across species at a global level would be advantageous when evaluating the utility of model organisms [30, 37-41]. For example, recent studies have demonstrated that only SL interactions involved in particular processes are conserved between yeast and human cancer cell lines, notably those involved in chromosomal instability [28], limiting the utility of such analysis. Other SL interactions that tend to be highly conserved include those whose protein products physically interact [37] or function in the same biological process (e.g. chromosome segregation) [42], suggesting these might be characteristic features of highly penetrant SLs.

Using computational approaches that identify highly penetrant SL interactions (Figure 4)

Whilst it is now relatively straightforward to identify potential SLs from LoF screens, the steps we have outlined above to distinguish highly penetrant from less penetrant SLs are both labour- and time intensive. Computational tools to prioritise the most promising candidates would therefore be of great value.

Filtering using prior knowledge. One approach to identifying highly penetrant SLs from a set of candidate interactions (e.g those identified in an isogenic screen) is to prioritise or filter these SL interactions using prior knowledge. Perhaps the simplest approach is to prioritise those candidate SLs previously observed in a different context - either with orthologous genes in a model organism [30, 42] or in distinct TCLs, as discussed above. The BioGRID database describes over half a million genetic interactions observed in non-human species [43], while the CancerGD resource details putative SLs identified in the majority of large-scale cell line panel screens published to date [44]. Such SLs, observed in different cell line panels or across different species, are likely to be strong candidates for highly penetrant SLs.

An attractive alternative to relying on existing genetic screen data is to use the wealth of 'omics' profiling data of tumours to identify or prioritise SLs. A number of methods have been developed to predict SLs from tumour molecular profiles and these could be extended to the task of identifying prioritising highly penetrant SLs. Perhaps the best-known approaches involve the identification of mutually exclusive genetic alterations - pairs of genes that are individually recurrently altered but altered in combination significantly less than expected by chance (Figure 3A). One interpretation of such mutually exclusive mutations is that the two genes may result in similar phenotypic outcomes when mutated and consequently there is no selective advantage to both genes being altered simultaneously. An alternative explanation is that the pair of genes are not mutated simultaneously because they display a synthetic lethal interaction. Although many computational approaches have

been developed to identify such mutually exclusive events [45], very few examples of mutually exclusive pairs have been experimentally demonstrated to reflect SLs [46].

A limitation to this approach is that both genes must be mutated at a relatively high frequency for the results to have any statistical significance. This makes it difficult to identify significant mutual exclusivity between genes that are not themselves driver genes. Jerby-Arnon *et al* [47] took an alternative approach and focussed on pairs of genes that were rarely both under-expressed in the same tumours, thus significantly expanding the set of genes for which mutually exclusive alterations could be identified. The recent availability of proteomic profiling data for tumours [48] opens up opportunities for the identification of mutually exclusive events using protein expression, e.g. searching for proteins whose expression is high when a tumour suppressor is defective [49].

Finally, it may be possible to develop heuristics to prioritise highly penetrant SLIs using our knowledge of the functions of individual genes and the physical and regulatory interactions between cellular components. These are discussed in **Text Box 1**.

Modelling approaches to predict SLs. A number of computational and mathematical approaches have been developed to model various cellular subsystems such as metabolism or signalling. These include flux balance analysis (FBA - used to model metabolic networks [50]) and approaches that describe regulatory and signalling networks using differential equations or boolean logic models [51]. In theory, all of these methods can be used to model the consequences of perturbations in individual genes as well as the effects of combined gene inhibition. However, in general, methods that are based on differential equations or logic models tend to describe the effect of gene perturbation on internal cellular states (e.g. the phosphorylation of a set of proteins, the expression of a particular gene) rather than cellular phenotypes such as proliferation or survival. In contrast, FBA has been used extensively to predict the impact of perturbations, including double gene deletions, on growth in a variety of systems [50, 52, 53]. To our knowledge it

is the only modelling approach that has been used to predict synthetic lethal interactions in human cancer (reviewed in [52]). A notable success in this regard was the identification of Haem oxygenase as a synthetic lethal partner of tumour suppressor fumarate hydratase [53].

FBA makes use of curated mathematical representations of cellular metabolism that describe the enzymes responsible for each metabolic reaction along with the metabolites produced and consumed by it. By making a number of assumptions, FBA can calculate the flow of metabolites through the represented network and ultimately predict the growth rate of a cell [50]. It is especially promising for the identification of highly penetrant SLIs because it can be used to model large numbers of combinatorial perturbations but also can be adapted to model different genetic backgrounds [54]. However, there are some major limitations of FBA. First, only a limited number of genes can be modelled using FBA (those whose function can be described in terms of reactions catalysed) and consequently predictions have not as yet been made for the majority of cancer driver genes. Secondly, the accuracy of the models when predicting SLIs is quite limited even for well characterised model organisms [50]. However, as our knowledge of metabolic networks expands so will the accuracy of the resulting predictions.

Extending the applications of highly penetrant SLs using guilt-by-association. One of the most striking observations from genetic interaction screens in model organisms has been the tendency of members of the same complex or pathway to display similar genetic interactions [55]. This suggests that if one member of a complex/pathway is a highly penetrant synthetic lethal effect with a particular gene, then there is a high probability that other members of the complex will be too. Computational approaches based on this **'guilt-by-association'** principal have been widely exploited to predict SLIs in model organisms [56]. As many distinct members of a pathway can be altered in different tumours, similar approaches could be applied to expand the applications of any highly penetrant SLs identified to additional driver genes. For example, the initial identification of synthetic lethality between defects in the *BRCA1/BRCA2* tumour suppressors and PARP inhibitors led to the

subsequent identification of SLs between PARP inhibitors and other tumour suppressor proteins that act in concert with BRCA1/BRCA2 in DNA repair [2, 57]. Similarly, the identification of a SLI between the SWI/SNF complex tumour suppressor ARID1A and the DNA repair kinase ATR, led to SLs between ATR and other SWI/SNF tumour suppressor genes being identified [58, 59] suggesting potential pathway level defects.

2D or not 2D - that, is the question

Many large-scale screens for synthetic lethality focus on the use of *in vitro* tissue culture (TC) approaches where cells are grown on an adherent surface. These "two-dimensional" *in vitro* TC approaches are often the preferred method as they provide a reproducible experimental workflow that is scalable to, for example, multi-well plate formats. What is not clear is how well these experimental formats replicate the complex, three-dimensional, structure of a tumour *in situ*, including cell-cell interactions that exist in a three-dimensional (3D) structure but not in two-dimensional (2D) TC. It seems possible therefore that some synthetic lethal effects identified in a 2D setting might be reversed in a 3D format (and *vice versa*), especially those that rely on mechanisms that involve cell-cell communication. One might think that those SL effects that operate in both 2D and 3D settings might be preferable to those that are private to either system. Likewise, for very pragmatic reasons, most large-scale synthetic lethal screens are *in vitro* screens and only involve one cell type. Again, it is possible that synthetic lethal interactions might be abrogated by, for example, tumour cell/stromal cell interactions. With these issues in mind, it is clear why all serious target validation efforts include an assessment of an *in vivo* model system that in some way attempts to replicate both the three-dimensional structure of a human tumour as well as an interaction with the stroma. In most cases, these types of *in vivo* analysis, such as the use of patient derived tumour xenografts (PDX), aren't yet routinely scalable to be used in the large-scale discovery of synthetic lethal effects, but the technical barriers preventing this will no doubt be rapidly overcome as improvements in *in vivo* CRISPR-Cas9 mutagenesis, mature. However, the use of these *in vivo* systems do play a key role in the assessment of penetrance. Furthermore,

where synthetic lethal interactions fail to be replicated in these models, some thought might be given to whether these ***stromal or three-dimensional synthetic rescue effects*** inform an understanding of the effects of cell-cell interaction.

Concluding remarks

The enthusiasm for exploiting the synthetic lethal principle to identify novel therapeutic targets in cancer is as high now as it has ever been. Many of the advances in gene manipulation technology have fuelled this enthusiasm, as has the large-scale delineation of essential gene lists in human tumour cells. However, we caution that technologies such as CRISPR-Cas9 screening will not be a panacea and an increased focus on discriminating highly penetrant synthetic lethal effects from those that are less penetrant is still required. Some methods already exist for estimating the penetrance of synthetic lethal effects and these could be enhanced by learning and then applying the principles that determine penetrance. Computational approaches to this question are emerging, which when combined with experimental methods, could be very effective.

Outstanding Questions (2000 characters, including spaces, required as separate word file using the designated heading)

- Which biological principles determine whether a synthetic lethal effect has complete or incomplete penetrance?
- Can these principles be determined and used to predict highly penetrant synthetic lethal effects?
- Can refined isogenic systems be developed that better model the spectrum of mutations present in human tumours?
- Can methods be developed that allow high-throughput perturbation screens to be performed *in vivo* at similar scale as *in vitro* perturbation screens?

- Can computational approaches replace or compliment empirical approaches for defining the penetrance of synthetic lethal effects?

Glossary (alphabetical)

Robustness: the extent to which a phenotype (e.g. synthetic lethality) persists in the face of other perturbations in a biological system

Stromal synthetic rescue effects: Where the activity of non-cancerous stromal cells reverses a synthetic lethal effect in a tumour cell, for example, by providing a cell survival signal to tumour cells

Synthetic lethality: where a combination, or *synthesis*, of gene defects causes cell death, but where individual gene defects do not

Synthetic lethal penetrance (SLP): the fraction of tumour cell clones with a specific genetic alteration (e.g. a cancer driver gene mutation) that undergo cell death when a synthetic lethal target is inhibited

Synthetic rescue: where a synthetic lethal effect is reversed, often *via* an additional molecular change

Three-dimensional synthetic rescue effects: Where the growth of tumour cells in a three-dimensional context reverses a synthetic lethal effect previously observed in two-dimensional tissue culture

Text Box 1. What features might highly penetrant synthetic lethal interactions have?

Analyses of genetic interactions in model organisms have identified that SLs with certain molecular properties are more likely to be conserved across species [38, 42, 60, 61]. For example, SLIs involving kinases and transcription factors are more poorly conserved across species than SLIs involving other gene types [61]. In contrast, SLIs involving pairs of genes whose protein products physically interact tend to be highly conserved [38, 42, 60]. These features have been used to develop computational models that predict conserved synthetic lethal interactions [42]. It may be possible to develop similar heuristics that distinguish highly penetrant effects from those that are less penetrant. The below characteristics are largely based upon first principles (and might at first glance appear self-evident), but where possible, we have exploited existing data to define what might characterise highly penetrant effects.

Characteristic 1 – inhibited process must be broadly essential. For a highly penetrant SLI, we predict that the combined perturbation of both genes disrupts a process that is essential for growth across many cell types (e.g. translation, protein degradation, DNA replication). If a process is only required in specific cell types than it is very unlikely that a SL which disrupts it will be highly penetrant. Alternatively, the process that is perturbed by a highly penetrant SL might not be essential in all cell types, but a commonly acquired characteristic (or hallmark [62]) of cancer, such as resistance to apoptosis.

Characteristic 2 – genes involved must function in related pathways. For a highly penetrant SL, we predict that the two genes involved in the SL should function in related biological processes. Often SLs identified in screens involve gene pairs with no obvious functional connection between them. Anecdotally these SLs seem less likely to replicate across multiple TCLs than SLs between gene pairs with a clear functional relationship (e.g. members of the same pathway). The closeness of the functional relationship between a

gene pair could be calculated as the shortest path between them on an integrated molecular interaction network, such as a protein-protein interaction network [4]. Such an approach would prioritise SLs involving pairs functioning in the same complex (i.e. those most conserved across species) or pathway over those functioning in distal processes.

Characteristic 3 – targeted genes must have not have highly cell type specific functions. For a highly penetrant SL, we predict that the targeted genes should have relatively constant functions across cell types, otherwise their perturbation is likely to have very variable phenotypic consequences across different tumour cell clones. Many transcription factors regulate the expression of different genes in different cell-types, suggesting that perturbing a transcription factor may not have similar phenotypic consequences across TCLs. In contrast, most subunits of the proteasome complex tend to form part of the proteasome in all cell types, suggesting their perturbation may have more fixed consequences across cell types. Distinguishing those genes that have fixed or variable functions across cells is non-trivial, but recent work suggests that analysing the transcriptional consequences of gene perturbation in different contexts may prove a useful proxy. Niepel *et al* [63] analysed the transcriptional profiles of multiple breast TCLs in response to perturbations and found that while the transcriptional response to certain drug classes (HSP90 inhibitors, cell cycle kinases) was relatively constant, other drug classes (MAPK inhibitors, PI3K inhibitors) caused cell-type specific transcriptional responses. There was some correspondence between the variability of the transcriptional response across cell lines and the variability of the phenotypic response.

Characteristic 5 – target genes should have few close paralogs. The protein to target therapeutically should have relatively few paralogs (gene duplicates) that might otherwise take over its role when its function is inhibited. Alternatively, the means of inhibiting the target (e.g. small molecule) should inhibit all of the target's paralogs also.

Characteristic 6 – synthetic lethal interactions should be conserved across species. It seems reasonable to think that SLs that are conserved across species barriers and are thus robust in the face of the extensive molecular re-wiring that accompanies speciation might be highly penetrant. Notably the BRCA/PARP synthetic lethality was observed in hamster, mouse and human cell lines [64-66].

Characteristic 7 – Mutual exclusive characteristics. It seems reasonable to think that when viewed in a large number of tumours, alterations in highly penetrant SL genes in tumours should be mutually exclusive. This mutual exclusivity could reflect an absence of synthetic rescue mechanisms and thus a highly penetrant SL.

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Figure legends

Figure 1. Synthetic lethality and synthetic lethal penetrance. A. Synthetic lethality. A synthetic lethal interaction, depicted by arrows, operates between two genes, A and B. Inactivation of either gene in isolation is compatible with cell viability and survival, whereas the combination, or synthesis, in defects in A and B causes cell death. **B.** Soft synthetic lethality. A synthetic lethal interaction exists between genes A and B but can be reversed by defects in either gene C, D or E. **C.** Hard synthetic lethality. In contrast to the soft synthetic lethality between A and B, the synthetic lethal interaction between genes F and G is only reversed by inactivation of H and is relatively resilient in the face of additional molecular alterations. **D.** Synthetic lethal penetrance. Genes A and B are synthetic lethal. The effect on cell inhibition caused by inactivation of gene B is shown for a fully penetrant effect (left) and an incomplete penetrant effect (right). **E.** Reasons for incomplete penetrance

Figure 2. Experimental approaches for the identification of synthetic lethal effects for a genotype of interest. There are three most common experimental approaches for identifying synthetic lethal effects using cell line models. **A.** High-throughput perturbation screens on a single isogenic system can be utilized for a given genotype of interest where Gene A is wild type (blue) or mutant (orange). **B.** Perturbation screens as in A could be performed on a panel of cell lines with extensive molecular heterogeneity annotated according to the status of the GoI where Gene A is wild type (blue, n = 10) or mutant (orange, n = 10). **C.** High-throughput perturbation screens on multiple isogenic system using different lineages and/or species representing extensive molecular heterogeneity, could be utilized for a given genotype of interest where Gene A is wild type (blue) or mutant (orange). **D.** Following the identification of a series of synthetic lethal effects (e.g. between Gene A and either B, C, D or E) iterative synthetic rescue screens could be performed where Gene A and B are simultaneously perturbed and high throughput functional screens could be performed to reverse the synthetic lethal effect between Gene A and B. The same approach is taken to rescue the synthetic

lethal effects between Gene A and C, D or E. The penetrance of a synthetic lethal effect is established by counting the number of molecular routes to synthetic rescue with the synthetic lethal effect between Gene A and E being demonstrated to be the most penetrant.

Figure 3. Computational methods to identify synthetic lethal effects. A. Mutually exclusive mutations may identify potential SLs. Genes A and C are mutated in a mutually exclusive fashion, suggesting a potential SL **B.** Flux balance analysis can be used to model metabolic activity. SLs can be identified by modelling how pairwise gene knockouts impact growth (centre), while synthetic rescue effects can be identified by modelling higher order gene knockouts (right) **C.** Guilt by Association. A hard SL (solid red line) involving subunits of distinct protein complexes potentially indicates additional SLs between other members of the two complexes (dashed red line) **D.** Shortest Path. SLs between genes with close functional relationships (e.g. genes A and B, which physically interact) may be more penetrant than those between genes with only indirect connections (e.g. A and E, which are 3 hops apart on the protein-interaction network).