Synthetic Lethality in Cancer Therapeutics

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Abstract

Treatment with targeted drugs has primarily focused on the genes and pathways that are mutated in cancer, which severely limits the repertoire of drug targets. Synthetic lethality exploits the notion that the presence of a mutation in a cancer gene is often associated with a new vulnerability that can be targeted therapeutically, thus greatly expanding the arsenal of potential drug targets. Here we discuss both the experimental and the computational biology tools that can be used to identify synthetic lethal interactions. We also discuss strategies for using synthetic lethality to discover new drug targets and in the rational design of more potent drug combinations. We review the progress made and future opportunities offered by synthetic lethal approaches to treating cancer more effectively.

1. THE CONCEPT OF SYNTHETIC LETHALITY

Drug treatment for cancer depends on the notion that mutations that give rise to the development of cancer also bring about a weakness that can be exploited therapeutically. Large-scale cancer genome sequencing efforts have catalogued mutations in various cancer types that can be explored as tumor-specific vulnerabilities (Weinstein et al. 2013). These genetic alterations consist of gain-of-function mutations in which genes are amplified, translocated, or mutated, and loss-of-function mutations in which gene function is compromised by missense mutation or deletion. The former group of mutations has been the subject of intense focus by the pharmaceutical industry for the development of targeted cancer drugs. These efforts have resulted in a number of cancer drugs that target activated driver oncogenes, such as *HER2*, *BCR-ABL*, *EGFR*, and *BRAF* (Pagliarini et al. 2015). These drugs target signaling proteins that are aberrantly activated as a direct consequence of an oncogenic mutation, and hence their inhibition is detrimental to the cancers. This dependence on oncogenic driver pathways is commonly referred to as oncogene addiction (**Figure 1*a***) (Weinstein 2002). From a drug discovery perspective, the loss-of-function mutations are much harder to tackle, and the same is true for a number of activated oncogenes that have proven to be more or less undruggable, such as the MYC transcription factor and the RAS proteins. Therefore, alternative strategies are required to target the vulnerabilities induced by these classes of cancer-causing genes.

Synthetic lethality provides the possibility of drugging undruggable targets indirectly. Synthetic lethality refers to a genetic principle in which the combination of two genetic perturbations is lethal, whereas each individually is not (**Figure 1*b***). Over the course of evolution, many redundancies and feedback loops in cellular signaling have developed to maintain cellular homeostasis when the extracellular milieu changes (Prahallad & Bernards 2015). Such redundancies in signaling ensure that cells can often survive when a single gene is inhibited because another gene can functionally compensate for it. However, inhibiting these compensatory genes may induce cell death specifically when the first gene is mutated, but not affect the growth of cells lacking this mutation. Likewise, when inhibition of a signaling pathway leads to biochemical activation of a

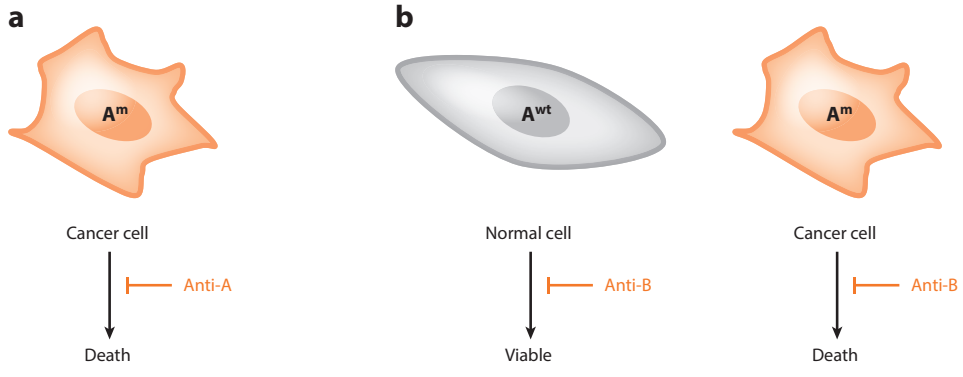

Figure 1

Oncogene addiction versus synthetic lethality. (*a*) Oncogene addiction. Cancer cells having an activating mutation in gene A will be addicted to the signal generated by the encoded gene product and are consequently hypersensitive to drugs that inhibit the pathway activated by A. (*b*) Synthetic lethality. When genes A and B are synthetic lethal, inactivation of gene B (either genetically or by a drug) will be lethal to cancer cells having a mutation in gene A but not to normal cells in which gene A is not mutated. Consequently, the inhibition of B is selectively lethal to cancer cells with mutations in gene A. Abbreviations: m, mutant; wt, wild type.

second pathway that mediates survival, simultaneous inhibition of both pathways can induce cell death due to a synthetic lethal interaction. It is unclear how many synthetic lethal gene pairs exist in human cells. Under standard culture conditions, some 2,000 genes appear to be essential to human cells (Blomen et al. 2015).

There are two important aspects to synthetic lethality in the context of cancer drug development. First, the genes that are synthetic lethal with oncogenic driver mutations are not necessarily mutated in cancer. Therefore, the exploitation of synthetic lethal interactions in cancer cells can potentially significantly expand the number of oncology drug targets. This concept is especially attractive if the driver mutation is undruggable. Cells that have lost a tumor suppressor gene may have gained an increased dependence on another gene, which may not be an oncogene. This phenomenon has been termed non-oncogene addiction (Solimini et al. 2007). Second, the effects of drugs that have no (or limited) clinical activity as single agents could be greatly potentiated when used in combination with a second drug that is synthetic lethal with the first drug. Here, we discuss applying the concept of synthetic lethality to the development of novel (and combinations of) cancer therapeutics.

2. CELL MODELS FOR STUDYING SYNTHETIC LETHALITY

The ability to carry out loss-of-function genetic screens in a massively parallel way, discussed in Section 3, has provided an avenue to systematically search for synthetic lethal interactions. In such genetic screens, one can use so-called isogenic cell line pairs—cells that differ in only a single mutation—to search for genes whose inactivation kills only the mutant cell (**Figure 2*a***). Alternatively, one could create for synthetic lethality screens an isogenic cell pair by restoring normal gene function in a cell that has a mutant gene (**Figure 2*b***). A major complication in finding synthetic lethal interactions by using isogenic cell lines is that the interactions are often

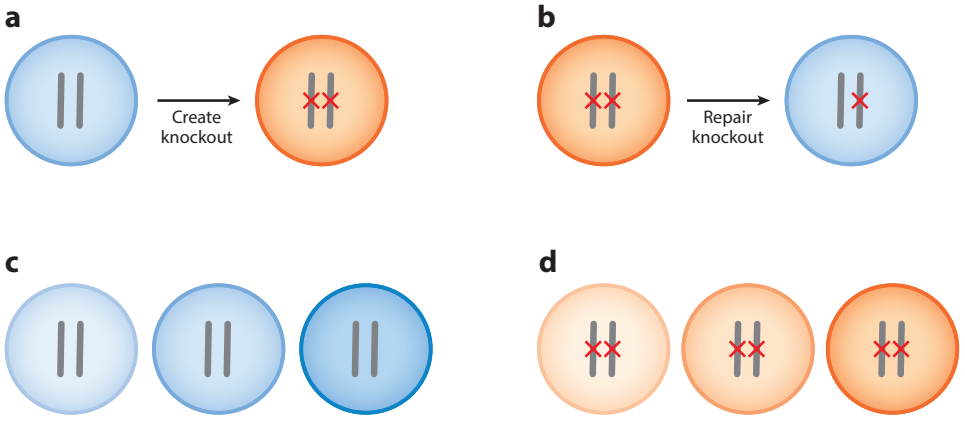

Figure 2

Cell models for studying synthetic lethality. (*a*) An isogenic cell pair can be created in which a given gene is inactivated through targeted mutagenesis. Performing synthetic lethality screens in wild-type (*blue*) and mutant (*orange*) cells allows for the identification of genes that are lethal only in the context of the targeted mutation. (*b*) Synthetic lethal screens can also be performed in isogenic cell pairs created by repairing at least one allele in a cell having two inactivated copies of a tumor suppressor gene and used for screening. (*c*,*d*) Having collections of cells that are all wild type for a given cancer gene and a second collection of cell lines that are all mutant for the same cancer gene allows for the identification of genes that are lethal to cells that have a cancer-specific mutation, independent of the cellular context.

context dependent, that is, they occur only in combination with other mutations or in a specific cell type or lineage. Thus, while the loss of gene X may be synthetic lethal with the loss of gene Y in one cancer, the presence of mutation Z may interfere with the synthetic lethal interaction between X and Y. As a consequence, a synthetic lethal interaction identified in any given cell pair may not be broadly valid, which limits its clinical utility. To avoid issues of context dependency, one could perform a series of genetic screens in panels of related cells that are wild type for the gene of interest (X in the example above) and in a second series of related cell lines that are all mutant for that same gene (**Figure 2*c***). Although this approach is time-consuming, searching for genes that are universally lethal in cells having a specific mutation versus those that do not avoids the context-dependency issue.

3. TOOLS FOR IDENTIFYING SYNTHETIC LETHALITY

The experimental determination of lethal interactions is based on identifying genes that, upon inactivation, show a lethal phenotype in the context of a specific genotype. Different methodologies exist that allow for perturbation of the expression of individual genes and can be used in large-scale screening. These include large libraries of synthetic short interfering RNAs (siRNAs), libraries of short hairpin RNAs (shRNAs), and, more recently, large collections of guide RNAs (gRNAs) for CRISPR/Cas9 genome editing. All these technologies can be applied in high-throughput screening formats in which each gene or each reagent is analyzed in individual wells or, with the exception of siRNA screening, can be used in a pooled format in which thousands of vectors are combined in one pool. Pooled screening quantitatively compares the relative abundance of individual shRNAs or gRNAs in the population before and after prolonged culture, either in the absence or presence of (drug) selection (Bernards et al. 2006, Shalem et al. 2014, Wang et al. 2014b, Zhou et al. 2014). Those shRNAs or gRNAs targeting genes that upon inactivation cause a lethal phenotype will be lost from the population (**Figure 3**). The abundance of each individual shRNA or gRNA can be determined by using high-throughput sequencing, and the fold change or depletion can be determined among the different populations. Pooled screening has the advantage that large collections of shRNAs or gRNAs can be interrogated with relative ease. This is important as multiple shRNAs or gRNAs per gene are needed because the efficiency of gene inactivation varies greatly for each shRNA or gRNA, and off-target effects associated with specific sequences can potentially cause false positives. Therefore, it is important that genes are selected as hits based on the behavior of multiple different shRNAs or gRNAs for a given gene. Several analytical tools are available that take the behavior of the different shRNAs or gRNAs targeting the same gene into account when generating a list of potential hits (Dai et al. 2014, Diaz et al. 2015, Li et al. 2014, Winter et al. 2016).

An important distinction between shRNA and CRISPR/Cas9 screens is that the latter can create full knockouts at the DNA level. As a consequence, gene inactivation is complete, whereas shRNA-based gene knockdown can vary greatly and is often incomplete. Although at first glance one would argue that complete knockdown should be preferred for the identification of synthetic lethal interactions, it is also possible that partial inhibition of an essential gene could create a (dosage) synthetic lethal phenotype in which the complete inactivation of the gene would be lethal by itself. In addition, one could argue that shRNA-mediated knockdown would more closely mimic the effect of drugs, as most drugs do not completely and continuously inhibit their target. Whether it is realistic to consider such genes as attractive therapeutic targets remains an open question. It is important to stress that shRNAs can also have significant off-target effects, complicating the interpretation of screening results. A potential solution to this problem is to create a knockdown with minimal off-target effects through the use of CRISPR interference or CRISPRi. This system exploits a catalytically inactive mutant CAS9 (dCAS9) fused to a KRAB transcriptional repressor

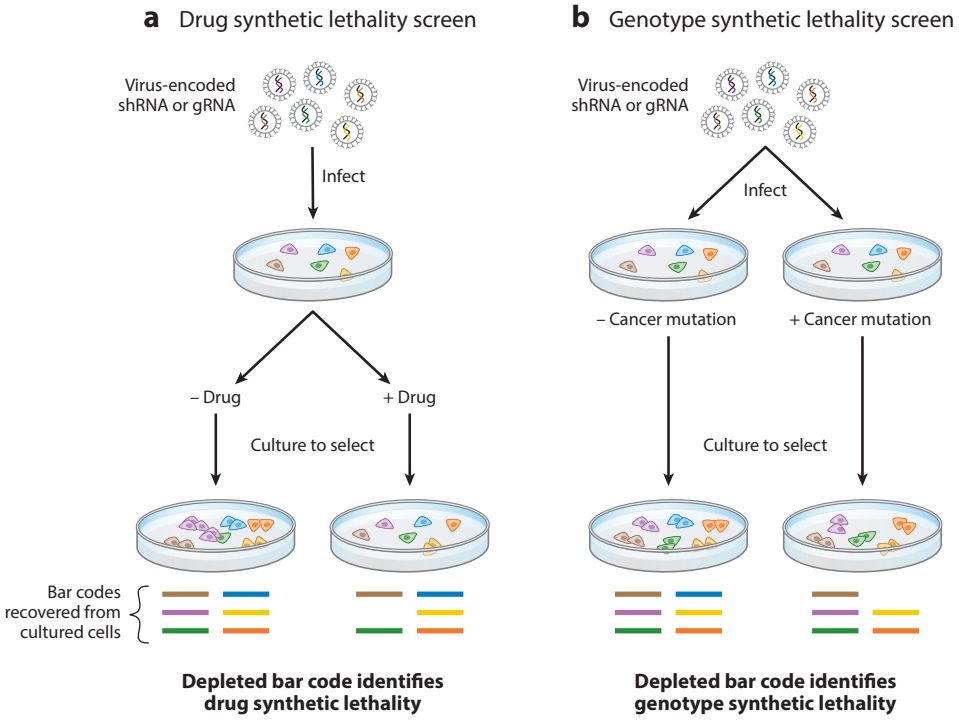

Figure 3

Finding synthetic lethal interactions through functional genetics. (*a*) Collections of short hairpin RNA (shRNA) or guide RNA (gRNA) vectors are introduced polyclonally into cells. Vectors that enhance sensitivity to the cancer drug will be depleted during drug selection. The presence of a bar code sequence allows for rapid identification of the depleted vectors through deep sequencing. (*b*) Collections of vectors are introduced into cells that have or lack a cancer-specific mutation. Vectors that are selectively toxic in cells having the cancer-specific mutation will become depleted compared with the reference population and can be identified through deep sequencing.

domain combined with gRNAs directed against the promoter region of a specific gene (Qi et al. 2013). CRISPRi can also be designed with inducible expression of either dCas9 or the gRNA. This allows for reversion of the knockdown phenotype but also facilitates the identification of essential genes in screens because the knockdown can be induced at a specific moment in a screen. It is to be expected that the design of specific and effective gRNAs targeting promoter regions will be improved and that CRISPRi will outperform shRNA screening for the identification of synthetic lethal interactions.

In the case of tumor suppressor genes, the identification of synthetic lethal interactions is based on the concomitant loss of the expression of two genes. When screening isogenic cell line pairs, a single gene of interest is tested against a large (genome-wide) collection of genes. However, it should also be possible to perform combinatorial screens in which thousands of pairs of genes are inactivated in a large population of cells. Indeed, several technology platforms, for both shRNA and CRISPR/Cas9, have been developed that allow for the simultaneous inactivation of two genes (or even more) in individual cells (Kampmann et al. 2014, Vidigal &Ventura 2015, Wong et al. 2016). Such combinatorial screens could generate genetic interaction maps for multiple different human cell types across large numbers of genes.

Haploid human cells have the advantage that a single insertional event can ablate gene function, and therefore, such cells could potentially be used for high-throughput synthetic lethality screens using insertional mutagenesis rather than gene-silencing vectors (Blomen et al. 2015). However, a disadvantage is the limited availability of haploid cell lines, with the risk of identifying cell line–specific or highly context-dependent synthetic lethal interactions.

4. APPLYING SYNTHETIC LETHALITY TO THE DEVELOPMENT OF CANCER THERAPEUTICS

4.1. Genotype-Selective Synthetic Lethality

Genotype-selective synthetic lethality takes advantage of the notion that the gain of a mutation by a cancer cell is almost invariably associated with a new weakness that can be targeted therapeutically. Such weaknesses can result from the inability to respond to a specific signal, such as DNA damage or cell cycle arrest, or the inability to maintain cellular homeostasis. The obvious advantage of this approach is that normal cells lack the mutation and, therefore, should not display the increased sensitivity to the synthetic lethal drug target. We discuss the progress made in identifying genotype-selective lethalities below.

4.1.1. *BRCA* synthetic lethality. Inherited loss-of-function mutations in *BRCA1* and *BRCA2* genes predispose to tumors of, primarily, the breast and ovaries (Venkitaraman 2002). Because *BRCA* gene products have a role in homologous recombination (HR) during repair of double-strand DNA breaks, it was hypothesized that inhibiting additional (partially redundant) DNA repair systems could be synthetic lethal with the loss of *BRCA* gene function. Indeed, inhibitors of the enzyme poly(ADP-ribose) polymerase (PARP), which has roles in base excision repair, were found to be strongly synthetic lethal with mutations in the *BRCA1* and *BRCA2* genes (Bryant et al. 2005, Farmer et al. 2005). Following positive clinical studies (Fong et al. 2009), use of the PARP inhibitor olaparib was approved for treating *BRCA*-mutated ovarian cancer in late 2014. The notion that HR-deficient tumors are sensitive to PARP inhibitors suggested that cells that have lost other enzymes involved in HR would also be hypersensitive to PARP inhibition. Indeed, deficiencies in the genes encoding RAD51, RAD54, DSS1, RPA1, NBS1, ATR, ATM, CHK1, CHK2, FANCD2, FANCA, or FANCC also conferred sensitivity to PARP inhibition (McCabe et al. 2006). Collectively, the phenotype resulting from a deficiency in HR is referred to as BRCAness, and it is likely that all cells displaying this phenotype will respond to PARP inhibition. As discussed in Section 4.1.7, it remains an open issue how such tumors can be most readily identified.

4.1.2. *RAS* synthetic lethality. The RAS proteins are the most frequently mutated and activated oncogenic drivers in human cancer. Large-scale sequencing has shown that activating *KRAS* mutations are present in 20% of non-small-cell lung cancers (NSCLCs), making *KRAS* an attractive drug target (Malumbres & Barbacid 2003). However, despite numerous attempts, identifying specific inhibitors has been challenging. Recently, compounds with promising activity have been described that specifically target the allosteric switch pocket of $KRAS^{G12C}$ (Ostrem et al. 2013, Patricelli et al. 2016). These small molecules further demonstrate that $KRAS^{G12C}$ rapidly cycles its nucleotide substrates, and this cycle can be modulated by upstream signals. Work by Patricelli et al. (2016) indicated that combining the inhibition of upstream signaling (e.g., EGFR inhibitors) with $KRAS^{G12C}$ was most effective in blocking both mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) signaling and inducing cell death. This is surprising,

considering that *KRAS*- and *EGFR*-activating mutations rarely co-occur and can even be synthetic lethal (Unni et al. 2015). These results suggest that RAS signaling must be delicately balanced for proliferation and survival. Because of the difficulty of targeting RAS proteins themselves, much attention has been devoted to inhibiting effectors downstream of RAS in the MAPK and PI3K pathways, including the RAF, MEK, and ERK protein kinases, as well as the PI3K and AKT kinases, and mTOR. Although many clinical trials of these drugs or combinations thereof are ongoing, it seems that these strategies only improve progression-free survival rather than overall survival.

One potential strategy is to find synthetic lethal interactions that occur with activated *RAS* oncogenes. Numerous efforts have been undertaken to identify genes displaying a unique dependency in mutant *RAS*-expressing cancer cells. One example is cyclin-dependent kinase 4 (CDK4), which is specifically required for *KRAS*-driven murine NSCLC. Surprisingly, the detrimental effect of CDK4 inactivation is not observed in lung cancer cell lines expressing wild-type *RAS* or other tissues expressing *KRAS* (Puyol et al. 2010). Ablation of *CDK4* in $KRAS^{G12V}$ lung tumors resulted in the induction of senescence and activation of an immune response, resulting in effective inhibition of tumor progression. The interaction between CDK4 and RAS is not restricted to NSCLC but has also been described for *NRAS*-mutant melanoma. In this melanoma model, inactivation of mutant *NRAS* results in proliferation arrest and apoptosis, which can be mimicked only by the combined pharmacological inhibition of MEK and CDK4 (Kwong et al. 2012). Although the interaction between RAS and CDK4 seems highly relevant, it could be considered an example of oncogene addiction rather than synthetic lethality, as CDK4 is downstream of RAS signaling pathways.

Several genetic screens have been performed to identify synthetic lethal interactions with mutant *RAS*, including panels of *RAS*-mutant and wild-type cancer cell lines and isogenic cell line pairs. These have yielded a large number of synthetic lethal interactions with mutant *RAS*, including the genes encoding for PLK1, STK33, TBK1, PKCδ, GATA2, TAK1, and CDK1 (for an extensive review, see Downward 2015). The study identifying PLK1 as synthetic lethal with *KRAS* mutation discovered that *RAS*-mutant cells are specifically sensitive to mitotic perturbations, as illustrated by enrichment of the genes associated with the anaphase-promoting complex (APC) and the proteasome, and that when inhibited they cause prometaphase accumulation and subsequent cell death (Luo et al. 2009). However, when comparing sensitivity to inhibitors of the proteasome or PLK1 across a large panel of cell lines, no clear correlation with *KRAS* mutations could be found (Barretina et al. 2012, Garnett et al. 2012). It is noteworthy that most synthetic lethal interactions with mutant *RAS* occur in those cancer cells that also depend on the continuous presence of mutant *RAS*. However, for several of these interactions it has been difficult to confirm the synthetic lethal interaction in the context of other cell lines, in in vivo models, or using small molecule inhibitors (Luo et al. 2012), and none of the interactions has proven effective in a clinical setting until now.

4.1.3. *MYC* synthetic lethality. The MYC family of transcription factors represents another group of interesting but elusive targets. *MYC* family genes, including *MYC*, *MYCN*, and *MYCL*, are frequently amplified, translocated, and overexpressed in many types of cancer, and they play a crucial part in proliferation, growth, and metabolism in most types of human malignancies. It has been demonstrated using genomic perturbations that the inactivation of MYC results in the loss of proliferation, the induction of differentiation, or even cell death through apoptosis (Shachaf et al. 2004, Soucek et al. 2008). Again, because of the inability to directly inhibit MYC using small molecules, it is important to identify synthetic lethal interactions with MYC. More than a decade ago, Wang et al. (2004) found that agonists of the TRAIL death receptor DR5 induced apoptosis in cells overexpressing the *MYC* oncogene, both in vitro and as tumor xenografts in

vivo. The mechanism underlying this phenotype is the upregulation of DR5 by MYC, which enhances the induction of apoptosis by DR5 agonists. As an extension of this work, an RNA interference (RNAi) screen for enhancers of TRAIL-induced apoptosis in MYC-overexpressing cells discovered that silencing GSK3β prevented phosphorylation of MYC on residue threonine 58, thereby preventing the recognition and subsequent degradation of MYC by the E3 ubiquitin ligase FBW7. The consequential upregulation of MYC results in increased levels of TRAIL DR5 and potentiation of DR5-induced apoptosis (Rottmann et al. 2005). Interestingly, these results demonstrate that MYC-expressing tumors could be treated with drugs that increase MYC expression and, thereby, leading to an acquired vulnerability to a second drug. Other examples of synthetic lethal interactions with MYC, but rather based on reducing MYC levels, are inhibition of the BET domain protein BRD4 (Delmore et al. 2011, Zuber et al. 2011) and depletion of Aurora A in *MYCN*-amplified neuroblastoma cells (Rottmann et al. 2005). The downregulation of *MYC* expression can also be the consequence of a synthetic lethal interaction itself, as exemplified by the interaction of the chromatin modifiers CBP (CREBBP) and p300 (EP300). Loss-of-function mutations in *CBP* are frequently found in lung cancer (15–20%), and a synthetic lethal RNAi screen found that *CBP*-deficient cancer cells were killed by the suppression of the paralog *p300*. The consequence of the loss of both proteins is downregulation of *MYC* expression and the induction of apoptosis (Ogiwara et al. 2016). Additional RNAi screens have identified synthetic lethal interactions of *MYC* with SUMO activating enzymes (SAE1 and SAE2); an AMPK regulator, AMPK-related kinase 5 (ARK5); and components of the core spliceosome, including BUD31, SF3B1, and U2AF1 (Hsu et al. 2015, Kessler et al. 2012, Liu et al. 2012).

4.1.4. *TP53* synthetic lethality. The function of the p53 tumor suppressor protein is lost in almost all cancers, either through mutation of the *TP53* gene or by alterations in components that control p53 activity. Given the role of p53 in response to cellular stress, DNA damage, and DNA repair, the loss of p53 creates vulnerabilities that can be explored as targets in the context of synthetic lethality. Indeed, several examples have been described associated with the DNA damage response. Normal cells undergo a p53-dependent G_1 arrest in response to DNA damage to allow for repair. In the absence of p53, cells strongly depend on the S and G_2/M checkpoints controlled by ATM, ATR, and the checkpoint kinases CHK1 and CHK2 to maintain genomic integrity. The inhibition of ATR or CHK1 can enhance the response of p53-deficient cells to DNA damaging agents, such as radiation, cisplatin, or doxorubicin (Wang et al. 1996). Similar synthetic lethal interactions have been found between p53 and the ATM/CHK2 pathway (Fedier et al. 2003, Nghiem et al. 2001). In addition, it has been found that the p38MAPK/MK2 pathway also can play a critical part in the DNA damage response of p53-deficient cells. Depletion of MK2 in p53-deficient cells, but not in p53 wild-type cells, causes mitotic catastrophe and the regression of tumors in vivo (Reinhardt et al. 2007). Using an elegant mouse model in which MK2-proficient and MK2-deficient cells are present in the same NSCLC, it was shown that MK2 contributes to the emergence of cisplatin resistance, which can be abrogated by MK2 loss or inhibition (Morandell et al. 2013). In addition to its role in DNA damage, the loss of p53 can also make cells more vulnerable to other type of stresses, such as metabolic stress. This is reflected in the dependency of p53-deficient breast cancer cells on PI5P4K and the observation that the upregulation of hexokinase 2, crucial for increased glycolysis, is required for cellular transformation and tumor formation in cells with the concurrent loss of PTEN and TP53 (Emerling et al. 2013, Wang et al. 2014a).

4.1.5. *RB1* synthetic lethality. Similar to TP53, the RB1 pathway is inactivated in the majority of human cancers, either through mutation of the *RB1* gene or by alterations in the pathways

that control pRB activation. Such genetic alterations include the loss of expression of *CDKN2A* (encoding $p16^{INK4A}$), overexpression or amplification of D-type cyclins, or mutations in the cyclin-dependent kinase CDK4. Reactivation of RB in cells that express wild-type RB can potentially be achieved by inhibiting the cyclin D–CDK4 or cyclin D–CDK6 complex. Indeed, a small molecule CDK4 and CDK6 inhibitor, palbociclib, is effective in *RB1* wild-type breast cancer (reviewed in Johnson et al. 2016, Sherr et al. 2016). In contrast to *TP53*, very few synthetic lethal interactions have been described for *RB1*. A genetic screen in *Drosophila* found that the loss of *RB* and *TSC2* induced cell death (Li et al. 2010). This lethal effect was also observed in human cancer cells in which the inactivation of *TSC2* specifically kills *RB1*-mutant cancer cells. *TSC2* loss activates TORC1, leading to a number of cellular stresses, including metabolic stress and oxidative stress. *RB1*-mutant cancer cells are not able to induce the reactive oxygen scavenger SOD2, leading to cell death induced by *TSC2* loss. A second example of a synthetic lethal interaction with *RB1* is the RB target *SKP2* (Wang et al. 2010). *RB1*-deficient human retinoblastoma cells undergo apoptosis after depletion of SKP2. SKP2 is a component of the SCF complex, where it acts as a substrate recognition factor and targets p27 for degradation. SKP2 is controlled by pRB at multiple levels: pRB binds SKP2 and, thereby, interferes with the binding and ubiquitination of p27; pRB–SKP2 binding facilitates SKP2 ubiquitination by the APC CDH1 ubiquitin ligase; and, lastly, SKP2 is a target for the E2F transcription factor. Thus, the loss of pRB results in increased amounts of SKP2 and decreased amounts of p27. When SKP2 is inactivated or p27 T187 phosphorylation is blocked, the loss of pRB results in cell death through apoptosis, thereby establishing synthetic lethality in susceptible cells (Emerling et al. 2013).

4.1.6. Synthetic lethalities of chromatin remodeling enzymes. The enzymes that are involved in the remodeling of chromatin are among the most frequently mutated genes in human cancer (Geutjes et al. 2012). Because many of these chromatin remodelers have enzymatic activity, the pharmaceutical industry has recently focused on developing small molecule inhibitors of these enzymes for their potential use in treating cancer. The SWI–SNF complexes appear to be frequently involved in human cancer, with mutations in 12–15 subunits occurring in roughly 20% of all human cancers (Helming et al. 2014a). In this complex, one of two ATPase subunits are present: SMARCA4 (also known as BRG1) and SMARCA2 (also known as BRM). Therefore, it was not surprising to find that *SMARCA4*-mutant tumors depend on a functional SMARCA2 protein for viability (Hoffman et al. 2014). Given that some 15% of lung adenocarcinomas have mutations in *SMARCA4*, selective SMARCA2 inhibitors could be highly specific agents for this group of cancers. A similar situation holds true for *ARID1A*, which is among the most frequently mutated chromatin remodelers. For instance, *ARID1A* is mutated in about half of all clear cell ovarian cancers (Jones et al. 2010). ARID1B is mutually exclusive with ARID1A in SWI–SNF complexes, and again, the loss of ARID1B was shown to be a specific vulnerability of *ARID1A*-mutant cancers (Helming et al. 2014b).

The two examples mentioned above are primarily of academic interest, as there are no selective inhibitors of SMARCA2 or ARID1B. From the therapeutic perspective, the finding that *ARID1A*-mutated cancers display synthetic lethality with the EZH2 methyltransferase is, therefore, of more significance (Bitler et al. 2015), as selective inhibitors for EZH2 are in clinical development and may be effective specifically in *ARID1A*-mutant cancers (Knutson et al. 2012). Another synthetic lethal interaction with potential clinical utility concerns tumors with loss-of-function mutations in the polycomb repressive complex 2 (PRC2). Inactivating mutations in the *SUZ12* component of the PRC2 complex are found in neurological cancers, and such tumors were found to be highly sensitive to bromodomain inhibitors, such as JQ1 (Filippakopoulos et al. 2010, De Raedt et al. 2014). Finally, evidence has indicated that tumors lacking histone H3 lysine 36 trimethylation

(H3K36me3) are selectively sensitive to inhibition of the kinase WEE1. Again, this may be directly relevant therapeutically, as the small molecule AZD1775 is a selective WEE1 inhibitor (Pfister et al. 2015).

4.1.7. Synthetic lethalities of cancer subtypes. The term convergent evolution refers to a process by which organisms can gain the same function through different routes. This process of convergent evolution also plays out in cancer evolution. For instance, *BRAF*^{V600E}-mutant colon cancers display a number of specific clinical properties, including poor prognosis upon relapse. Colon cancers having this *BRAF* mutation (some 10% of colon cancers) also have a distinctive gene expression signature (Popovici et al. 2012, Tian et al. 2012). However, this same gene signature is also shared with a group of colon cancers that lack a *BRAF* mutation but display the same clinical behavior as those that have a *BRAF* mutation (Popovici et al. 2012, Tian et al. 2012). Apparently, this gene signature can be acquired in multiple ways, only one of which is through the gain of a *BRAF* mutation. Collectively, tumors that display this gene signature are referred to as *BRAF*-like, and they represent some 20% of colon cancers. Vecchione et al. (2016) used an shRNA-based synthetic lethality screen in *BRAF*-mutant colon cancer cells to identify specific vulnerabilities in this group of tumors. They selected 363 genes that are upregulated in *BRAF*-like tumors as candidate synthetic lethal genes, arguing that these genes may be required to tolerate the *BRAF*-like state. It was found that only the loss of *RANBP2* was synthetic lethal with the presence of the *BRAF*-like gene signature, irrespective of the presence of the *BRAF* mutation. *RANBP2* loss induces defects in microtubule dynamics during mitosis in *BRAF*-like colon cancer cells, a process that is also disrupted by a group of cancer chemotherapeutics called vinca alkaloids. It turned out that all *BRAF*-like tumors were highly sensitive to the vinca alkaloid drug vinorelbine (Vecchione et al. 2016). These findings highlight the notion that a gene signature can be used to identify a group of tumors that has a specific vulnerability that can be exploited therapeutically.

As mentioned above, BRCAness is likely associated with sensitivity to PARP inhibition, but how can such tumors best be identified when no *BRCA* mutation is present? It has been found that *BRCA1*-mutant breast tumors have a distinctive pattern of genomic copy number aberrations (CNAs) (Wessels et al. 2002). Again, this pattern was also found in tumors lacking a *BRCA* mutation, making this pattern of CNAs a candidate biomarker for BRCAness. Indeed, in a retrospective study, breast tumors having this CNA pattern turned out to be highly sensitive to agents that induce double-strand DNA breaks, a known vulnerability of *BRCA1*-mutant tumors (Vollebergh et al. 2011).

A final example of a gene signature that may identify a specific vulnerability of a group of cancers relates to microsatellite instability (MSI) in colon cancer. MSI colon cancers are characterized by a high mutation load, lymphocytic infiltrate in the tumors, and a good prognosis. Recently, this group of tumors has been shown to be responsive to checkpoint immunotherapy, most likely because they are highly antigenic owing to their mutation load (Le et al. 2015). Using gene expression analyses, Sun et al. (2012) identified a gene signature that distinguishes these MSI colon cancers, but again found that a group of colon tumors exists that does not have MSI when assessed by clinical assay, but does have MSI when assessed by gene signature. Interestingly, this group of MSI-like tumors also is associated with a good prognosis and a higher mutation load than microsatellite-stable colon cancers. Therefore, it is plausible that this group, too, will benefit from checkpoint immunotherapy.

4.2. Synthetic Lethal Drug Combinations

Ever since the first use of chemotherapy for treating human cancers in the late 1940s, it has been observed that single-agent therapies deliver only transient benefit to patients owing to the

development of resistance (Farber & Diamond 1948). This limitation was slowly, but steadily, overcome by meticulous experimentation with combinations of chemotherapeutic drugs. It is depressing to realize that most of our progress in treating cancer with drugs has come from such trial and error approaches in which drugs are combined haphazardly without much scientific rationale. Seventy years later, we are witnessing a similar issue with the use of single-agent targeted cancer drugs, which also deliver benefits primarily in terms of progression-free survival, but hardly at all in overall survival, owing to the rapid development of drug resistance (Berns & Bernards 2012). A factor that contributes to the limited benefits of inhibiting a single signaling pathway is that signaling pathways tend to be highly interconnected. This cross talk between signaling pathways represents a major impediment to treating cancer, as cancer cells are programmed to readjust when a cancer drug inflicts a pathway perturbation. For the treatment of cancer, it is of particular importance to know which alternative pathways maintain cell viability when a major signaling pathway is inhibited by drug treatment. Unraveling the question of how the major signaling pathways are interconnected in different cell types seems daunting, but loss-of-function genetic screens represent a powerful tool for asking which signaling pathways are essential to a cancer cell in the presence and in the absence of a specific cancer drug. One salient example of this is the case of *BRAF*-mutant colon cancer. It had been shown as early as 2002 (Davies et al. 2002) that the *BRAF* gene is frequently activated by mutation in melanoma. This observation led to the development of highly selective, small molecule BRAF inhibitors that were subsequently shown to be effective in the treatment of *BRAF*-mutant melanoma (Chapman et al. 2011). Based on this, a trial was undertaken in *BRAF*-mutant colon cancers. Quite unexpectedly, this trial was negative (Kopetz et al. 2015). Apparently, the genotype alone is not always a good predictor of responses to targeted cancer drugs, and the context in which these mutations occur does seem to matter. To ask whether any pathway in *BRAF*-mutant colon cancer compensates for BRAF inhibition, Prahallad et al. (2012) carried out a synthetic lethality screen using an shRNA library targeting all kinases. They found that inhibiting EGFR is synthetic lethal with BRAF inhibition in colon cancer, suggesting a rational combination therapy for this disease. Based on these data, multiple clinical trials using this combination of inhibitors have been started, some of which have already reported positive results (van Geel et al. 2014).

RAS proteins link growth factor signaling to downstream pathways, including the RAF/MEK/ERK pathway (also known as MAPK pathway) and the PI3K pathway. Given the undruggable nature of RAS proteins, drug development efforts have focused on the kinases in the pathways downstream of RAS, including the MEK kinases. However, in both preclinical models of cancer and clinical trials, the results obtained with these MEK inhibitors in *KRAS*-mutant tumors have been disappointing (Adjei et al. 2008, Jänne et al. 2013, Migliardi et al. 2012). To address these results, synthetic lethality screens have been carried out to search for enhancers of MEK efficacy in *KRAS*-mutant tumors. In a kinome-centered screen, HER3 was identified as a synthetic lethal partner in NSCLC and in colorectal cancer (Sun et al. 2014). In a second loss-of-function synthetic lethality screen, BCL-XL was identified as synthetic lethal with MEK inhibition (Corcoran et al. 2013). For both synthetic lethal partners identified, experimental drugs are available, and clinical trials combining MEK with pan-HER inhibitors or BCL-XL inhibitors are in progress.

As a final example of synthetic lethal drug combinations, the Lars Zender group has focused on enhancing the response of hepatocellular carcinoma (HCC) to the multikinase inhibitor sorafenib. Patients with advanced HCC treated with sorafenib had a median overall survival of 10.7 months versus 7.9 months in the placebo group, making synthetic lethal drug combinations with sorafenib an attractive combination therapy (Llovet et al. 2008). Using a small library of shRNA vectors targeting genes that are amplified in HCC, MAPK14 was found to sensitize HCCs to sorafenib, suggesting a potential combination strategy (Rudalska et al. 2014).

A similarly poor response to monotherapy with a targeted agent was seen for the treatment of colon cancer and squamous cell carcinoma of the head and neck with EGFR antibody drugs (Cunningham et al. 2004, Vermorken et al. 2008). Because EGFR is hardly ever mutated in these cancers, the tumors are not truly addicted to EGFR signaling, providing a potential explanation for the limited clinical benefit that patients experience from EGFR therapy. Other examples are the modest benefit of antiangiogenic therapy with bevacizumab in breast cancer. This lack of effectiveness has resulted in the withdrawal of marketing approval by the US Food and Drug Administration (FDA) for using this drug to treat breast cancer. This highlights the urgent need to find more effective combinations of these drugs to deliver more clinical benefit to patients. Synthetic lethality screens using these drugs may help identify more powerful drug combinations.

5. IN SILICO PREDICTION OF SYNTHETIC LETHALITY

Because the search for synthetic lethal interactions, within cancer and diseases in general, is a daunting experimental task, many approaches have been proposed to perform this prediction in silico. Here, we discuss a selection of these approaches and classify them into four broad categories.

5.1. Semimechanistic Pathway Models

Semimechanistic pathway models represent the first category of in silico prediction, consisting of approaches that focus on specific pathways or processes in the cell, which are invariably determined by the cancer type and the associated driver genes. These approaches build semimechanistic models of pathways based on knowledge as well as tailor-made experimental measurements. Although these models are limited in scope, they aim to capture the more intricate wiring patterns within and between a small number of pathways. Once such models have been constructed, they can be interrogated in silico for synthetic lethal interactions. For example, Klinger et al. (2013) applied a variant of modular response analysis to (re)identify the feedback activation of EGFR upon MAPK inhibition in colorectal cancer. Similarly, logic modeling was employed to detect synthetic lethal interactions in AGS gastric cancer cells (Flobak et al. 2015). The simulations predicted five synergistic interactions, four of which were also experimentally validated. These include known MEK–AKT or MEK–PI3K interactions, along with novel combinations involving TAK1–AKT and TAK1–PI3K.

5.2. Metabolic Network Models

Metabolic network models consist of approaches that specifically employ metabolic maps (Duarte et al. 2007) and interrogate these via combinatorial in silico perturbation for synthetic lethal and synthetic dosage lethal interactions. In a synthetic dosage lethal pair, the overactivity of one partner renders the other essential (Sajesh et al. 2013). In metabolic network approaches, the effect of the knockout of a reaction in a metabolic network representing normal cells is typically contrasted with the effects of the same knockout in a network constructed or modified to represent cancer cells. To model the latter, high-throughput molecular data, such as data on gene expression, are employed to identify metabolic reactions that are aberrantly affected in a given cancer type (Facchetti et al. 2012, Folger et al. 2011, Megchelenbrink et al. 2015).

5.3. General Network Models

The third category encompasses approaches that perform in silico mining for synthetic lethal interactions on large networks (or combinations of these) of various kinds. These approaches

include DrugComboRanker, which integrates disease networks and drug functional networks and then exploits the joint structure of the disease and drug networks to identify drugs that could potentially synergize owing to their targeting patterns in the disease network (Huang et al. 2014). Similarly, TIMMA (Target Inhibition Interaction using Maximization and Minimization Averaging) employs drug-target networks derived from affinity measurements and combines these with large-scale cell line drug screens to identify combinations of targets and the associated drug combinations that will likely synergize (Tang et al. 2013). In a similar vein, Zhao et al. (2011) developed a machine-learning approach to predict synergistic drug combinations by employing STITCH (Search Tool for Interacting Chemicals), a drug-target interaction network, and combining this with properties (features) of the drug set of interest, such as targets and indications. They detect feature patterns (combinations of features) that are enriched in approved drug combinations, are predictive for new drug combinations, and also provide mechanistic insights regarding combinatorial therapy. Not surprisingly, pairs of proteins targeted by approved drugs are predictive for synergy in new drug combinations. For the predicted synergistic combinations, 69% were supported by the literature, and the remainder represent potentially novel drug combinations (Zhao et al. 2011).

5.4. Large-Scale Data Mining

The final category is home to approaches that perform data mining on large-scale data sets based on universal principles of synthetic lethality and, specifically, on how this would manifest in the molecular patterns selected for in cancer cells. In contrast to the approaches described for other categories, these approaches do not rely on prior knowledge captured in pathway maps or networks. The DIGRE (Drug-Induced Genomic Residual Effect) computational model is the approach that fared best in the 2014 DREAM challenge, aimed at predicting synergistic drug combinations (Bansal et al. 2014). This approach employs gene expression profiles of cell lines in the presence of a collection of drugs and then uses the notion of drug-induced gene expression profiles to predict synergy. Specifically, the hypothesis is that the combinatorial effect of compounds A and B results from the residual genomic changes induced by one of the drugs prior to the application of the second. Recently, the Genomics of Drug Sensitivity in Cancer panel of 1,001 cancer cell lines screened against 265 drugs was employed to develop LOBICO (Logic Optimization for Binary Input to Continuous Output), an approach to predict responses to single drugs based on molecular data. LOBICO is a logic-based model that selects a small number of molecular features (<4) and combines these in a logic formula to predict drug response. For example, cell lines are predicted to be responsive to MEK inhibitors if they harbor either a *RAS* or a *RAF* mutation or, more formally: "IF *RAF* mutated OR *RAS* mutated THEN sensitive" (Iorio et al. 2016). More generally, such models can be employed to predict drug synergies in the following way: If mutations in genes A and B predict sensitivity for drug Ai targeting A, then Ai will most likely synergize with Bi, a drug targeting B, given that B is an inactivation alteration.

Arguably one of the most comprehensive, large-scale, in silico efforts to identify synthetic lethality resulted in an approach referred to as DAISY (data mining synthetic lethality identification pipeline) (Jerby-Arnon et al. 2014). DAISY employs three inference strategies to detect synthetic lethality. The first strategy is known as genomic survival of the fittest and is based on the principle that there is strong selection against the loss of both genes constituting a synthetic lethal pair. DAISY, therefore, mines molecular profiles of cancer cell lines and tumors for pairs of inactivating events that occur less frequently than expected by chance, in other words, mutually exclusive events. The second strategy is shRNA-based functional examination, and it is based on the principle that in cells in which one partner is absent, deletion of the second is not tolerated. This is implemented by looking in shRNA screens for associations of genomic alterations (deletions or inactivations)

that coincide with hairpins dropping out. The third and final inference strategy identifies pairs of genes that show pairwise coexpression, and it is based on the principle that synthetic lethal gene pairs typically participate in the same process and, therefore, show coexpression.

In addition to searching for synthetic lethality, DAISY also searches for synthetic dosage lethality. To construct a final ranked list of synthetic lethal pairs, DAISY traverses all gene pairs (approximately 534 million) and examines every pair to determine whether it fulfills each one of the three criteria described above. Gene pairs that fulfill all three criteria in a statistically significant manner are predicted to be synthetic dosage lethal pairs. DAISY was tested on synthetic lethal interactions that had been experimentally determined for cancer specifically to identify the synthetic lethal partners of PARP1, the tumor suppressors *VHL* and *MSH2*, and the synthetic dosage lethal partners of the oncogene *KRAS*. DAISY was evaluated using 7,276 gene pairs that had been experimentally tested in six large-scale screens (Bommi-Reddy et al. 2008, Lord et al. 2008, Luo et al. 2009, Martin et al. 2009, Steckel et al. 2012, Turner et al. 2008). DAISY outperformed competing approaches by reaching an overall area under the curve of 0.78. The survival of the fittest strategy was the strongest contributor to prediction accuracy, and adding co-expression as a requirement further improved the predictions. Interestingly, shRNA-based functional examination was not predictive of synthetic lethality on its own. However, it was predictive of synthetic dosage lethality. Experimental validation of the *VHL* synthetic lethal partners showed an almost fourfold increase in true hits compared with a similar competing screen (Bommi-Reddy et al. 2008). Finally, the targeted inhibition of nine DAISY-predicted *VHL* synthetic lethal partners with FDA-approved drugs validated six synthetic lethalities, as their inhibition reduced cell growth. Notably, none of these drugs is currently employed to treat cancer, but they are approved for other conditions, such as hypertension and depression.

6. CLINICAL APPLICATIONS OF SYNTHETIC LETHALITY

The only drug that is approved for clinical use based on a synthetic lethal interaction is PARP inhibitors for the treatment of *BRCA*-mutated ovarian cancer. However, quite a few clinical trials are ongoing that take advantage of synthetic lethal interactions identified in the laboratory. Most of these trials are based on drug-drug synthetic lethality, rather than on the genotype-specific synthetic lethality seen between *BRCA* mutation and PARP inhibition. Based on the synthetic lethal interaction between BRAF and EGFR inhibitors described above, a number of trials are under way, including National Clinical Trial numbers 01719380, 01750918, and 01791309. There are also multiple trials based on the synthetic lethal interaction between MEK inhibitors and pan-HER inhibitors in *KRAS*-mutant cancers, including National Clinical Trial numbers 02039336, 02230553, and 02450656. As discussed in the next section, we foresee a significant potential for using synthetic lethality genetic screens to identify drug combinations and to find specific vulnerabilities in cancers of a defined genotype. To identify such synthetic lethal interactions, it will be important to understand the cross talk between signaling pathways, which often confounds simple genotype–drug-response relationships. As discussed above, a major effort is still needed to map the signaling feedback and cross talk circuits in cancer cells to identify the interdependencies between signaling pathways, which will be instrumental to expediting the rational design of synthetic lethal drug combinations (Bernards 2012).

7. SUMMARY AND FUTURE PERSPECTIVES

As described in Section 6, the application of the concept of synthetic lethality has not yet resulted in major benefits for patients with cancer. First, a major reason for this is that the use of this

concept is relatively new in oncology and that drug development is a slow process. A second factor hampering clinical translation is the notion that synthetic lethal interactions can be highly context dependent, as discussed in Section 4.1.2 for vulnerabilities associated with *KRAS* mutation. A lack of understanding of the context dependencies precludes selection of patients for a given synthetic lethal drug combination. A third factor may be that shRNAs have abundant off-target effects, which can complicate interpretation of data obtained through large-scale genetic screens to identify synthetic lethal interations. It appears that CRISPR and CRISPRi are superior in their selectivity for the intended target, which will likely result in more reliable data from genetic screens (Evers et al. 2016). Finally, a more fundamental problem may be that cell line models are not reliable tools for identifying synthetic lethal interactions that are relevant for patients with cancer. We consider this to be unlikely. Cell responses to perturbations are often hard-wired, which makes such responses relatively insensitive to environmental signals. These cell-autonomous responses are also most likely to be context independent. Recently, the use of three-dimensional tumor organoids was suggested as a powerful alternative to the use of two-dimensional cell line models (Van De Wetering et al. 2015). It should be borne in mind that such organoids also depend on artificial culture conditions, which makes it questionable whether their use in synthetic lethality screens is superior over conventional cell lines. The use of an in vivo genetic screen in genetically engineered mouse models of cancer appears attractive, but these models are not suitable for high-throughput genetic screens as have been performed in vitro.

A major societal concern in the treatment of cancer is the price of new agents. Pharmaceutical industries have always justified the high prices of cancer drugs by citing the high attrition rates of candidate cancer drugs during the clinical development process (Hay et al. 2014). Indeed, in 2013 alone, some 40 drugs were dropped from the global oncology pipeline, many because they lacked single-agent efficacy (Williams 2015). Such lack of single-agent efficacy may have its origins in the redundancy and feedback loops that exist between the major signaling pathways in cancer. Consequently, inhibiting only a single pathway may not yield sufficient therapeutic benefit to improve on the current standard of care. This notion implies that some potentially useful cancer drugs may have been abandoned prematurely because they were not tested in the right combination. As discussed above, BRAF inhibition in *BRAF*-mutant colon cancer requires combinations with an EGFR inhibitor, whereas single-agent BRAF inhibition is effective in *BRAF*-mutant melanoma. Synthetic lethality genetic screens provide an unbiased approach for identifying powerful combinations of drugs that may have been dropped from development owing to the lack of single-agent activity. A systematic synthetic lethal screening of such abandoned drugs may yield a treasure trove of combination therapies and, thereby, reduce attrition rates, which in turn should have a favorable effect on drug pricing.

Another major issue in drug treatment is the development of resistance. Conventional cancer therapies rely on standard protocols in which a given agent is designated as a first-line therapy, which can be followed by second- and third-line therapies in case of resistance. In general, such later therapies are less effective than first-line therapy, but do they need to be? If we consider the notion that each strength gained by a cancer comes with a weakness that can be exploited therapeutically, then we can view drug resistance as a new strength that is accompanied by a new weakness. Synthetic lethality screens in drug-resistant cells, therefore, hold the promise of uncovering novel and potentially greater vulnerabilities in a drug-resistant cancer. Such greater vulnerabilities, when identified, could in turn result in more effective second-line therapies rather than in less effective therapies, as is currently mostly the case. The task of identifying acquired vulnerabilities for each individual drug-resistant cancer seems daunting. However, in contrast to resistance to chemotherapy, the number of ways in which cancers can develop resistance to targeted cancer drugs is limited. For instance, *EGFR*-mutant lung cancers treated with EGFR

inhibitors develop secondary mutations (*EGFR*^{T790M} in some 50% of cases) (Kobayashi et al. 2005). Similarly, treating *BRAF*-mutant melanoma with BRAF or MEK inhibitors, or both, results in reactivation of the RAS/RAF/MEK/ERK pathway in the majority of cases (Van Allen et al. 2014, Wagle et al. 2014). Thus, when drug-resistance development becomes predictable, so does the new vulnerability that is associated with that resistance. Hence, we may learn in the coming years which mechanisms of drug resistance induce which new therapeutic opportunities. Once we understand the sequence by which new vulnerabilities arise in cancer, we may be able to treat cancer sequentially with therapies that are at least as effective as, or even more effective than, the first-line therapy. This, together with recent developments in immuno-oncology, may make the title of the recent book by DeVita & DeVita-Raeburn (2015), *The Death of Cancer*, become a reality sooner rather than later.

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