OPEN
ACCESS

Drugging the addict: non-oncogene addiction as a target for cancer therapy

Remco Nagel[†], Ekaterina A Semenova[†] & Anton Berns[*]

Abstract

Historically, cancers have been treated with chemotherapeutics aimed to have profound effects on tumor cells with only limited effects on normal tissue. This approach was followed by the development of small-molecule inhibitors that can target oncogenic pathways critical for the survival of tumor cells. The clinical targeting of these so-called oncogene addictions, however, is in many instances hampered by the outgrowth of resistant clones. More recently, the proper functioning of non-mutated genes has been shown to enhance the survival of many cancers, a phenomenon called non-oncogene addiction. In the current review, we will focus on the distinct non-oncogenic addictions found in cancer cells, including synthetic lethal interactions, the underlying stress phenotypes, and arising therapeutic opportunities.

See the Glossary for abbreviations used in this article.

Introduction

With around 15 million new cases per year, and 8 million deaths related to this disease, cancer is one of the leading causes of death worldwide [1]. Most commonly, tumors that cannot be eradicated by surgery or radiotherapy due to metastatic dissemination or the risk thereof are combatted using therapeutics interfering with one of the hallmarks of cancer that distinguishes them from normal tissues [2]. For example, tumor cells generally are more sensitive to DNA-damaging agents and compounds interfering with mitotic progression compared to their non-transformed counterparts, mostly due to their increased proliferation rates [3,4]. Even decades after their introduction into the clinic, many of these compounds are still the standard of care for many tumor types. Some cancers, such as testicular germ cell tumors, show remarkable remissions or even complete eradication after the administration of DNA-damaging agents, proving that these therapies can be highly effective [5]. In contrast to this, rapid development of resistance to genotoxic compounds is observed in many other tumor types, such as small-cell lung cancer, ovarian cancer, and head-and-neck cancer [6–8]. In addition, the administration of these drugs is accompanied by severe side effects, mostly related to damage inflicted on proliferative normal tissues, such as hematopoietic precursors and gastrointestinal mucosal cells [9]. These limitations of currently used chemotherapeutics underscore the need for novel cancer cell-specific drug combinations that provide high efficacy with limited side effects.

Several tumor cell types critically depend on the continuous activation of oncogenic signaling for their survival, a phenomenon termed "oncogene addiction" (Fig 1A) [10]. This finding has led to the development of several therapies designed to inhibit the activated oncogene, which was hypothesized to eradicate tumor cells containing enhanced oncogenic signaling while sparing the normal tissue. The introduction of Herceptin (trastuzumab), an antibody that inhibits the *HER2* oncogene in breast cancer, showed that this strategy was extremely effective against *HER2*-dependent cancers [11]. This success was soon followed by the development of inhibitors targeting several other oncogenes, such as the *BCR-ABL* fusion gene in chronic myeloid leukemia [12], KIT in gastrointestinal tumors [13], EGFR and ALK in non-small-cell lung cancer [14,15], and *BRAF* in melanoma [16]. Unfortunately, even though in many instances remissions were observed, this was frequently followed by the reappearance of drug-resistant tumor clones.

In addition to the complications caused by drug resistance, the strategy of targeting oncogene addiction can only be applied to a limited number of cases. This stems from the fact that most mutations found in tumors, including some oncogene activating mutations and all loss-of-function mutations, are not directly druggable. The abundantly activated oncogenes *RAS* and *MYC*, for example, have been shown to be essential for tumor cell survival in *in vitro* and *in vivo* models harboring active forms of these genes and are prime examples of oncogene addiction [17,18]. Unfortunately, it appears difficult to identify compounds that are able to efficiently inhibit these specific oncogenes. Similarly, no compounds are available that can restore the loss of function of tumor suppressor genes. To overcome this problem, it was proposed that anticancer treatment should be focused not only on directly counteracting the oncogenic mutations, but also on the altered dependencies of cancer cells on non-mutated genes [19]. This proposal is based on the genetic studies in *Drosophila* that showed genetic incompatibility

Division of Molecular Genetics, The Netherlands Cancer Institute, Amsterdam, The Netherlands
*Corresponding author. Tel: +31 20 512 1991; E-mail: a.berns@nki.nl
[†]These authors contributed equally to this work

Glossary

ABL	ABL proto-oncogene 1, non-receptor tyrosine kinase
ALK	Anaplastic lymphoma receptor tyrosine kinase
ALL	Acute lymphoblastic leukemia
APC/C	Anaphase-promoting complex/cyclosome
ATM	Ataxia telangiectasia mutated
ATP	Adenosine 5'-triphosphate
ATR	Ataxia telangiectasia and Rad3-related
AURKA	Aurora kinase A
AURKB	Aurora kinase B
BCR	BCR, RhoGEF, and GTPase-activating protein
BRAF	B-Raf proto-oncogene, serine/threonine kinase
BRCA1	Breast cancer 1
BRCA2	Breast cancer 2
BUD31	BUD31 homolog
CDC6	Cell division cycle 6
CDK1	Cyclin-dependent kinase 1
CDK2	Cyclin-dependent kinase 2
CDK	Cyclin-dependent kinase
CHK1	Checkpoint kinase 1
DDR	DNA damage response
DNA	Deoxyribonucleic acid
DR5	Death receptor 5
E2F	E2F transcription factor
EGFR	Epidermal growth factor receptor
EIF4EBP1	Eukaryotic translation initiation factor 4E binding protein 1
EIF4E	Eukaryotic translation initiation factor 4E
FBXW7	F-box and WD repeat domain-containing 7
GATA2	GATA binding protein 2
GSK3B	Glycogen synthase kinase 3B
GST	Glutathione S-transferase
HER2	Erb-b2 receptor tyrosine kinase 2
HIF1	Hypoxia-inducible factor 1
HRAS	Harvey rat sarcoma viral oncogene homolog
HR	Homologous recombination
HSET	Kinesin-related protein HSET
HSF1	Heat-shock factor 1
HSP70	Heat-shock protein 70
HSP90	Heat-shock protein 90
KIF2C	Kinesin family member 2C
KIFC1	Kinesin family member C1
KIT	KIT proto-oncogene receptor tyrosine kinase
KRAS	Kirsten rat sarcoma viral oncogene homolog
MAPK	Mitogen-activated protein kinase
MED4	Mediator complex subunit 4
MPS1	Monopolar spindle 1
MTH1	MutT-type nudix hydrolase 1
mTOR	Mammalian target of rapamycin
MYCL	v-myc avian myelocytomatosis viral oncogene lung carcinoma-derived homolog
MYCN	v-myc avian myelocytomatosis viral oncogene neuroblastoma-derived homolog
MYC	v-myc avian myelocytomatosis viral oncogene homolog
NBS1	Nijmegen breakage syndrome 1
NFE2L2	Nuclear factor, erythroid 2-like 2
NFκB	Nuclear factor kappa B
NHEJ	Non-homologous end joining
NLK	Nemo-like kinase
NRAS	Neuroblastoma RAS viral oncogene homolog
NRF2	Nf-E2-related factor 2
NUDT1	Nudix hydrolase 1
PARP	Poly-(ADP-ribose) polymerase
PDK1	Pyruvate dehydrogenase kinase 1
PI3K	Phosphatidylinositol-3-kinase
PIP4K2	Phosphatidylinositol-5-phosphate 4-kinase type 2
PLK1	Polo-like kinase 1
PLK4	Polo-like kinase 4
PNKP	Polynucleotide kinase 3'-phosphatase
PRKDC	Protein kinase DNA-activated catalytic polypeptide
PTEN	Phosphatase and tensin homolog
RAD51	RAD51 recombinase
RAS	Rat sarcoma viral oncogene homolog
RB1	Retinoblastoma 1
RHEB	RAS homolog enriched in brain
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SAE1	SUMO-activating enzyme 1
SAE2	SUMO-activating enzyme 2
siRNA	Short interfering RNA
SKP2	S-phase kinase-associated protein 2
SNAI2	Snail family zinc finger 2
SODs	Superoxide dismutases
TP53	Tumor protein p53
TRAIL	TNF-related apoptosis inducing ligand
TSC2	Tuberous sclerosis 2
WNT	Wingless type
WT1	Wilms tumor 1

between mutations referred to as synthetic lethality. Synthetic lethality describes the phenomenon of a lethal combination of perturbations in two genes, where mutation in either of those genes alone does not affect cell viability (Fig 1B) [20]. Reasoning along those lines, it was therefore proposed that targeting a synthetic lethal partner of a mutated driver gene in tumor cells may allow for therapeutic intervention that would spare normal cells [19].

In the search for synthetic lethal interactors that could be exploited for cancer therapy, it was also observed that tumor cells often display increased levels of various stresses. The tumorigenic process induces re-wiring of many processes and leads to augmented cellular stress levels, such as increases in DNA damage and replication stress, metabolic stress, and proteotoxic and oxidative stress [21,22]. These stress phenotypes have been observed in many distinct tumor types, and even though it is not completely understood how some of them originate, their induction appears to be tightly linked to oncogenesis. Different tumors have been shown

to critically depend on the reduction of these stress levels for their survival. To exploit this cancer-specific stress phenotype, two approaches have been proposed that would affect tumor cells while sparing normal cells. On the one hand, inhibition of stress-reducing pathways would be expected to increase the levels of stress specifically in tumor cells to critical levels. On the other hand, applying a stress overload can specifically kill cancer cells as they possess less buffering capacity in comparison with normal cells [21]. Therefore, re-sensitizing tumor cells to their increased stress levels or generating imbalances in these levels by application of additional stress has been put forward as a promising means for therapeutic intervention [23].

The survival of cancer cells thus depends on a multitude of factors that distinguish them from non-transformed cells, which include but are not limited to oncogenic drivers. In contrast to normal cells, tumor cells might rely on the constitutive function of genes that are synthetic lethal partners of driver mutations. In
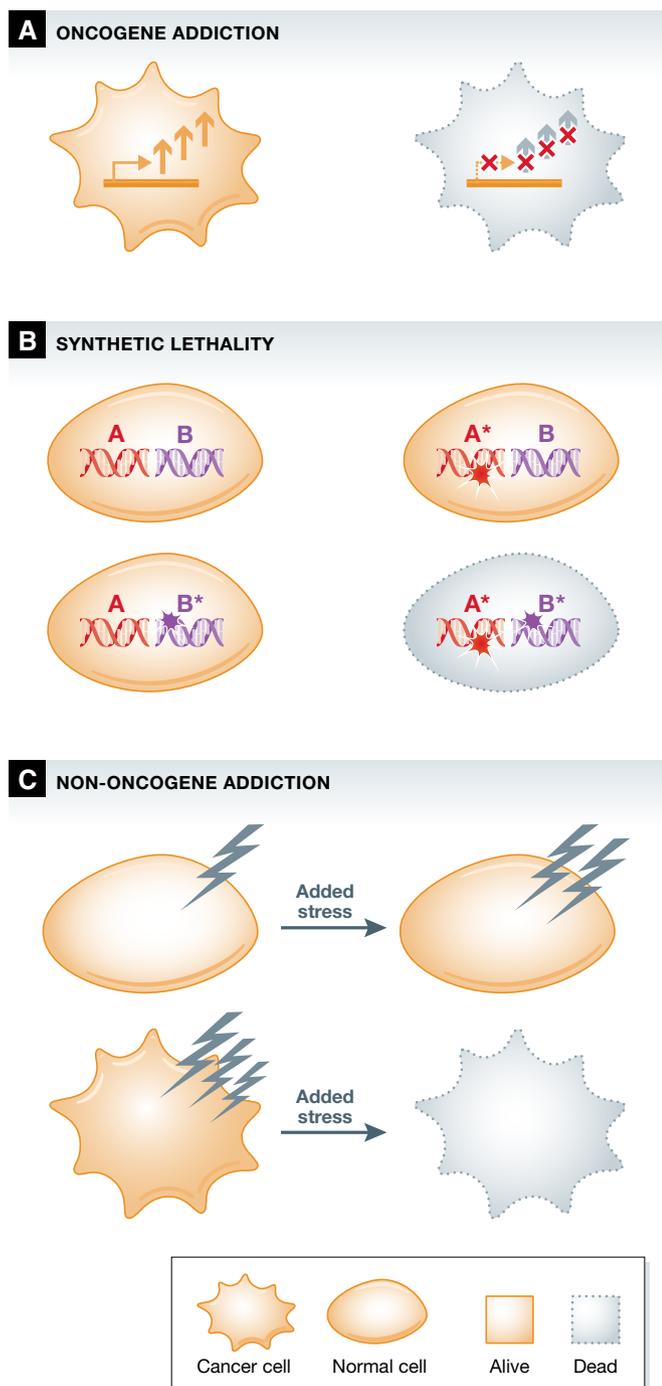
Figure 1. Schematic representation of cellular effects of oncogene addiction, synthetic lethality, and non-oncogene addiction.
(A) Oncogene addiction. Cancer cells need continuous oncogenic signaling for their survival. Increased oncogenic signaling in the cancer cell is schematically represented by the arrows. (B) Synthetic lethality. The mutation of individual genes is compatible with cell viability, whereas the combined mutation of these genes leads to cell death. (C) Non-oncogene addiction. Cancer cells harbor elevated levels of various stresses, caused by collateral events during the tumorigenic process. Tumor cells can be specifically killed by application of additional stress, or by inhibition of specific salvage pathways, whereas normal cells can tolerate these perturbations.

addition, they also rely on pathways that are able to buffer the increased stress levels that are encountered. As these genes and pathways are generally not contributing to oncogenesis itself, the dependency on their proper function has collectively been dubbed "non-oncogene addiction" (Fig 1C) [23]. As most synthetic lethal partners of oncogenic driver genes are not contributing to tumorigenesis, but are essential for tumor cell survival, synthetic lethality can be regarded as a specific subtype of non-oncogene addiction. In the following section, currently known non-oncogene addictions and the stress phenotypes they relate to will be described. Next, the specific synthetic lethal partners of driver genes frequently mutated in cancer will be discussed, with a specific emphasis on the stress phenotypes they are affecting.

Non-oncogene addiction

Proteotoxic stress

Many of the dependencies on stress relief have been identified by forward genetic screens in cell lines harboring mutations frequently found in human cancers. Several of these genomewide screens aiming to identify genes essential for cancer cell survival yielded multiple hits in genes involved in proteasomal degradation and protein folding [24–26]. Even though components of these processes have generally been regarded to be essential for any cell, a wealth of accumulated data suggest a higher dependency of cancer cells on these genes for their survival.

In vivo knockdown of HSF1, the master regulator of the heat-shock response that protects cells from protein misfolding and aggregation showed this protein to be essential for tumor initiation and maintenance. HSF1 is induced by various protein-denaturing stresses and, among others, regulates the expression of the chaperones HSP70 and HSP90 [27,28]. Somatic mutations in *HSF1* have not been recorded in human tumors and increases in *HSF1* gene expression are unable to transform cells, suggesting that the protein itself is not causative for tumorigenesis. In contrast, chemically and genetically induced tumor formation was greatly affected in *Hsf1* knockout mice, leading to reduced tumor incidence and tumor volumes in the absence of HSF1 [29]. Strikingly, *Hsf1* is dispensable for growth and survival in the absence of acute stress, which indicates that the dependency of cancer cells on *Hsf1* is a vital non-oncogene addiction [29,30].

Tightly linked to the dependency of cancer cells on increased protein folding capacity is their dependency on proteasomal degradation. It has been shown that genetic aberrations can lead to an elevated level of proteotoxic stress. Aneuploidy, copy number variations, and transcriptional alterations affect the dosage of components of protein complexes and thereby promote the formation of protein aggregates [31–33]. Furthermore, specific gene mutations leading to improperly folded proteins are an additional source of increased proteotoxic stress [34]. This elevated stress level presumably needs to be reduced via an upregulation of proteasomal degradation and protein folding capacity to allow for survival. Indeed, upregulation of *HSF1, HSP70*, and *HSP90* and increases in proteasomal degradation are common events in human cancers [27,35]. In addition, upregulation of autophagy has been shown to be another means for tumor cells to reduce proteotoxic stress as it poses an alternative route to clear damaged or aggregated proteins.

Augmented autophagy is observed in many cancers, suggesting that tumor cells highly depend on this process to alleviate the proteotoxic stress they encounter [36,37]. Interestingly, inhibition of these antistress responses shows promising anticancer activity, demonstrating the efficacy of exacerbation of the proteotoxic stress encountered by tumor cells [22,38].

For currently unknown reasons, *KRAS* mutant tumors are more sensitive to the proteasome inhibitor bortezomib [39]. Additionally, cancers bearing specific *BRAF* or *EGFR* mutations have an increased sensitivity toward HSP90 inhibition, most likely due to these proteins being specific clients of this chaperone [40]. Altogether, these findings indicate that targeting of non-oncogene addiction such as inhibition of the proteasome or heat-shock response proteins could well serve as precision treatment of cancers.

Oxidative stress

Cancer cells generally suffer from higher levels of oxidative stress than non-transformed cells [41]. The increase in this stress level can be directly caused by oncogenic signaling, which induces elevated levels of ROS [42,43]. Collectively, the higher ROS levels deliver oxidative damage to all macromolecules in the cell, where the most detrimental effects accumulate as mutations in the DNA, leading to genomic instability. The double-stranded DNA itself, however, is thousands-fold less susceptible to oxidative damage than the free nucleotides it is composed of [44]. Two recent studies showed that cancer cells critically depend on the removal of oxidized nucleotides from the total pool of free nucleotides for their survival. Both studies indicated that the function of the normally non-essential gene *NUDT1* (also known as *MTH1*) is essential for tumor cell survival [45,46]. Inhibitors of *MTH1* selectively killed cancer cells, indicative of their need to reduce the damage inflicted on nucleotides by increased levels of oxidative stress.

In addition to MTH1, also NFE2L2 (better known as NRF2) plays a major role in keeping oxidative stress levels in cancer cells in check. NRF2 is a stress-induced transcription factor that regulates the expression of many cellular defense enzymes, including antioxidant genes such as the *SOD*s and *GST* [47,48]. Knockouts for *Nrf2* are more prone to develop chemically induced tumors, suggesting that this gene functions as a tumor suppressor and inhibits tumor initiation [49,50]. In various sporadic tumors, however, NRF2 levels are drastically upregulated and high expression of this protein is a marker of poor prognosis [51–54]. These contradictory findings are likely to stem from the antistress function of NRF2. Whereas the presence of NRF2 initially protects against the mutational damage inflicted by ROS and thereby has a negative impact on tumor initiation, in later stages it promotes malignant progression by reducing oxidative stress levels that transformed cells suffer from [55]. Thus, whereas NRF2 normally functions as a tumor suppressor, many cancer types eventually become addicted to the expression of this gene in order to survive with their increased ROS levels.

Oxidative stress overload through inhibition of antioxidant proteins could be used to therapeutically target cancer cells, as it reduces the ROS buffering capacity of cells. Similarly, agents that enhance ROS production would be hypothesized to exert the same effect. This approach has already shown promising results in preclinical models, where the ROS-inducing agent piperlongumine elicited potent antitumor effects. In contrast to the major effects it exerted on cancer cells, the compound only showed limited toxicity toward primary non-transformed cells [56]. In addition, other compounds that induce ROS production, such as dichloroacetate and beta-phenylethyl isothiocyanate, have shown similar anticancer effects [57,58]. Collectively, these studies suggest that the generation of an imbalance in oxidative stress levels could be a potent strategy to combat cancer.

Metabolic stress

Most normal cells metabolize glucose to carbon dioxide via mitochondrial respiration to generate energy for all cellular processes. In contrast, the large majority of cancers have been shown to switch to an alternative route for their glucose metabolism, glycolysis [59]. Coinciding with this change in metabolism, an increased glucose uptake has been observed in the majority of tumors [60]. Cancer cells seem to benefit from this switch in metabolism as it results in the formation of intermediate products that can subsequently be used for the generation of fatty acids, nucleotides, and amino acids necessary to support the continuous growth of the tumor [61,62].

Multiple cellular factors play a role in the upregulation of glycolysis, with hypoxia-inducible factor 1 (HIF1) being one of the main regulators for this switch. In addition to *HIF1*, oncogenic activation of *RAS* and the *PI3*-kinase pathway or the inactivation of the tumor suppressor *TP53* can also induce a switch to glycolysis [63–65]. Mechanistically, enhanced signaling through these pathways results in elevated levels of glucose transporters on the cellular surface thereby causing a glucose-induced inhibition of mitochondrial respiration and stimulation of glycolysis [66–68].

Indications that the reliance of tumor cells on an altered glucose metabolism could be used for therapeutic intervention came from studies that inhibited the glycolytic process. When glycolysis was suppressed, tumor cells were unable to sufficiently upregulate mitochondrial respiration, which eventually resulted in cell death [69]. Administration of the non-metabolizable glucose analogues 2-deoxyglucose or 3-bromopyruvate inhibited glycolysis and induced apoptosis specifically in cancer cells with compromised mitochondrial respiration [70–72]. Strikingly, 3-bromopyruvate elicited remarkable reduction in the size of xenografted tumors, without affecting normal tissue [71]. Similarly, inhibition of PDK1, an important signaling kinase downstream of HIF1, triggered apoptosis in tumor cells both *in vitro* and *in vivo* [73–75].

In addition to a change in glucose metabolism, changes in amino acid metabolism and the dependency thereon have also been observed in cancer cells. It has, for example, been shown that some rapidly proliferating cancer types have an increased reliance on the production or uptake of the non-essential amino acids arginine, asparagine, glycine, glutamine, and serine [67,76–79]. *MYC*-driven tumors are particularly sensitive to glutamine withdrawal, because genes involved in glutamine metabolism are under direct transcriptional control of this oncogene [80–82]. *RAS*-driven tumors were shown to depend on macropinocytosis, an endocytic process that leads to internalization of extracellular molecules, for their glutamine supply. The availability of glutamine for these cells can be reduced by pharmacological inhibition of the macropinocytic uptake, which was shown to be effective in targeting RAS-transformed cells [83]. Additionally, specific cancer types are more sensitive to the deprivation of essential amino acids when compared to non-transformed cells. Whereas normal cells enter a cell cycle arrest upon leucine deprivation, a wide array of melanoma cell lines

undergoes apoptosis in the same situation. This differential response was shown to be caused by a failure of melanoma cells to activate autophagy, which would normally be able to liberate molecules, such as leucine, from internal sources [84].

Inhibition of folate and nucleotide metabolism with agents such as methotrexate and pemetrexed already resulted in clinical benefit [85]. This suggests that depletion of essential metabolic end products can specifically target tumor cells and would be a means for therapeutic intervention. The direct deprivation of nutrients from the tumor microenvironment to induce tumor cell death *in vivo* has proven to be difficult. Nonetheless, the administration of L-asparaginase, a bacterial enzyme that deaminates and thereby destabilizes asparagine, has significantly improved the outcomes for ALL [86]. ALL cells are particularly sensitive to limited supplies of asparagine as they cannot produce sufficient levels of this molecule to sustain their metabolic demands [87]. In addition, the observations that whole-body metabolic alterations, such as obesity and diabetes, can have profound effects on cancer development have led to studies on the effects of dietary changes on tumor progression [88,89]. Strikingly, when mice bearing *TP53*-mutated tumors were fed a diet lacking serine, tumor growth was significantly impaired due to a rapid suppression of glycolysis [77]. Similarly, a fasting protocol was able to inhibit tumor growth *in vivo*, where the delay in progression was comparable to the one seen when classical chemotherapeutics were administered [90]. This response was suggested to be a direct result of the incapability of tumor cells to switch to a sufficiently high level of oxidative phosphorylation in environments containing low glucose levels [91]. Alternatively, the effects of glucose deprivation could also be mimicked by AMPK activation using metformin, which is able to elicit cell death specifically in *TP53*-mutated cells [92]. Altogether, these studies indicate that interference with the altered metabolism in cancer is a promising avenue for tumor cell-specific targeting.

Centrosome clustering

Centrosomes nucleate and organize the microtubule skeleton necessary for equal distribution of sister chromatids to the daughter cells. During each cell cycle, these organelles are duplicated only once, ensuring the proper establishment of a bipolar spindle with both centrosomes serving as poles [93]. It has been observed, however, that many tumor cells contain an elevated number of centrosomes, which originate from overduplication or failure of cytokinesis [94,95]. Interestingly, loss of the tumor suppressors *TP53, RB1, BRCA1,* or *BRCA2* or the expression of the human papillomavirus oncoproteins E6 or E7 can all lead to centrosome amplification, suggesting a causal role for some tumorigenic events in this overduplication [96–100].

An amplified number of centrosomes generally induce multipolar cell division, which is detrimental to cell survival as it leads to mitotic catastrophe [101]. Strikingly, this multipolarity can be prevented by centrosomal clustering at the poles, which generates a pseudo-bipolar spindle that allows for normal division [102,103]. The molecular mechanism behind this clustering has not yet been fully elucidated, but proteins that involved the spindle assembly checkpoint, acto-myosin contractility, kinetochore and microtubule attachment, the chromosome passenger complex, sister chromatid

cohesion components, and microtubule-associated proteins have all been shown to play a role in this process [104,105]. As cells with supernumerary centrosomes rely on their clustering for proper cell division, inhibition of these proteins has been proposed as a means to specifically target cancer cells. Indeed, inhibition of the microtubule-associated motor protein KIFC1 (better known as HSET) leads to the declustering of centrosomes and elicits death specifically in cells with amplified centrosomes [104,106]. Along these lines, several compounds have been identified that induce centrosome declustering leading to cancer cell death, while leaving non-transformed cells unaffected [107–110]. Centrosome declustering to disrupt the mitotic balance in cancer cells thus emerges as a promising anticancer strategy, which currently awaits further clinical validation.

DNA damage stress/replication stress

It has been long recognized that impaired DNA damage repair can lead to tumorigenesis as it can result in an accumulation of mutations, amplifications, deletions, and chromosomal rearrangements. This increase in genomic instability is considered to be a driving force for transformation, as elevated levels of DNA damage are already observed in the earliest stages of the development of various cancers [111]. Accidental lesions occurring in proto-oncogenes and tumor suppressor genes may trigger this. In line with this, many tumor suppressors function in different DDR pathways and their loss severely compromises the capacity to repair damaged DNA, which can even lead to a severe predisposition to the development of cancer in the case of inherited genetic mutations in DDR genes. These predisposing mutations have been identified in well-known tumor suppressors, such as *ATM, NBS1, BRCA1,* and *BRCA2,* and the Fanconi anemia genes [112]. In addition to loss of tumor suppressors leading to elevated DNA damage levels, the activation of many oncogenes can also enhance the level of genomic instability by the induction of replication stress. In particular, elevated levels of MYC can induce replication stress through induction of hyper-replication and unscheduled firing of extra origins of replication [113]. This can ultimately result in the collapse of replication forks and generate double-stranded DNA breaks.

For decades, the interference with DNA damage stress levels has been used as a strategy to combat cancer, and it is still the mainstay for treatment of many tumor types. Ionizing radiation and DNA-damaging agents take advantage of the high genomic instability of cancer cells by overloading their stress defense system. The development of several targeted therapies that inhibit proteins involved in DDR pathways has brought novel opportunities to generate imbalances in the DNA damage stress load in cancer cells. Interference with the function of the main sensor kinase for replicative stress, *ATR*, seems to be an especially powerful method to specifically target *MYC*-induced cancers. For example, *MYC*-driven tumorigenesis could be prevented by genetic ablation of *ATR* in both lymphoma and pancreatic mouse models [114,115]. In addition, MYC overexpression seems to induce sensitivity to inhibitors of ATR and CHEK1, the downstream relaying kinase of ATR [114,116]. These preclinical studies suggest that destabilization of replication forks by inhibition of the ATR signaling axis could prove to be valuable in the targeting of cancer cells that harbor oncogene-induced replicative stress.

Synthetic lethality

BRCA–PARP

The presence of a multitude of DNA damage repair pathways, such as HR, NHEJ, and base excision repair, ensures that non-transformed cells can cope with the genomic insults they encounter. Interestingly, when tumor cells lose functionality of one specific repair pathway, they seem to become highly dependent on the remaining repair pathways for their survival [117]. This observation has led to the development of specific therapies that exploit a synthetic lethal vulnerability in two individual DDR pathways. The paradigm synthetic lethal interaction between the HR and NHEJ is currently being used to treat BRCA-deficient breast and ovarian cancers [118]. The loss of BRCA1 or BRCA2 function leads to impaired HR, creating a critical dependency on NHEJ for the repair of double-stranded breaks in the DNA. PARP inhibitors are able to abrogate the NHEJ pathway, and administration of these inhibitors results in the accumulation of unrepaired DNA and eventually elicits cell death specifically in BRCA-deficient tumors [119]. Similar to this, any tumor deficient in HR would be expected to be vulnerable to PARP inhibition, which is why current investments are being made to identify more markers for HR deficiency.

PTEN

The *PTEN* gene is one of the most frequently inactivated tumor suppressors in human cancer [120]. It has been long recognized that one of the main functions of PTEN is to dephosphorylate phosphatidylinositol 3,4,5-trisphosphate leading to the formation of phosphatidylinositol 4,5-bisphosphate. Hereby PTEN antagonizes the activity of the highly oncogenic PI3K pathway, which promotes cell survival, proliferation, growth, and cellular metabolism [121]. More recently, a novel function of PTEN was observed in studies on the genomic instability that is displayed by *Pten* knockout mice. As it was previously thought that PTEN's function was being executed in the cytoplasm, it came as quite a surprise that these novel studies showed an additional role for PTEN in the nucleus. PTEN was shown to directly act on the chromatin, and deletion of the gene reduced the transcription levels of Rad51, an essential recombinase involved in HR [122]. The reduction of Rad51 levels in *PTEN*-deficient cells leads to a fivefold decrease in the capacity to repair DNA double-strand breaks via HR, thereby decreasing genomic stability [123].

Considering the different functions of PTEN, the specific targeting of *PTEN*-deficient cells has been approached via two routes. On the one hand, inhibition of mTOR, a direct downstream target in the PI3K pathway, is preferentially toxic to *PTEN*-deficient cells, underscoring the oncogenic addiction of *PTEN*-mutated cells to the PI3K pathway [124,125]. On the other hand, synthetic lethal partners of PTEN have been identified which have an impact on the elevated levels of DNA damage that result from the loss of this protein. Similar to *BRCA*-deficient cells, *PTEN*-deleted cells exhibited a decreased capacity for HR, and are thereby extremely sensitive to the inhibition of PARP [123,126,127]. Strikingly, rescue experiments making use of mutant forms of PTEN showed that the phosphatase-inactive forms of this protein were still able to give resistance to PARP inhibitors. In addition, impairment of the nuclear localization of functional PTEN leads to sensitivity to PARP inhibition, indicating that the sole determinant of sensitivity to these inhibitors is the nuclear function of PTEN [123]. Analogous to their dependency on PARP,

PTEN-deficient cells are also relying on the function of PNKP for their survival [128]. PNKP participates in multiple DNA repair pathways, and the dependency of *PTEN*-deficient cells on this protein therefore reinforces the finding that these cells highly depend on DNA repair pathways other than HR for their survival [129].

Tightly linked to the dependency on DNA repair, *PTEN*-null cells are also highly sensitive to inhibition of checkpoint kinases. Specifically, these synthetic lethal interactions were identified with a variety of mitotic spindle kinases, which normally respond to aberrant mitoses upon DNA damage. The synthetic lethal partners for PTEN in this group of kinases include NLK, PLK4, and MPS1 [130,131]. Inhibition of these kinases in *PTEN*-deficient cells specifically led to aberrant division and mitotic catastrophe, suggesting that these cells rely on this checkpoint due to their increased level of DNA damage stress [131]. More recently, ATM was brought forward as a synthetic lethal partner for PTEN. Inhibition of ATM in *PTEN*-null cells led to many mitotic abnormalities [132], suggesting that both ATM and the mitotic checkpoint kinases are essential to prevent mitotic catastrophe in these cells.

In summary, all these studies suggest that the loss of PTEN leads to an oncogenic addiction to the PI3K pathway, as well as to salvage pathways that deal with the increased DNA damage stress induced by the loss of this gene. Many of the described synthetic lethal interactions can be exploited therapeutically, as a wide range of inhibitors are available for all these kinases and DNA damage repair proteins.

TP53

Aberrations in *TP53* are found in about 50% of all tumors making it the most frequently mutated gene in cancer [133]. The wild-type p53 protein is stabilized upon DNA damage, after which it exerts its protective function to allow for repair of the damaged DNA [134]. Due to the cytoprotective function, this gene is considered as one of the guardians of the genome, and hence, its loss allows for the accumulation of other mutations needed during the carcinogenic process [135]. TP53 performs its functions via multiple mechanisms, including the establishment of a cell cycle arrest in G1/S-phase, activation of DNA repair pathways, and induction of apoptosis [133].

As cells that lack functional p53 are unable to instigate a DNA damage-induced G1 arrest, they seem to heavily rely on a functional G2/M cell cycle checkpoint for their survival. This is illustrated by the identification of synthetic lethal interactions of *TP53* with a variety of mitotic kinases, including *CDK1*, *PLK1*, *AURKA*, and *WEE1*. Inhibition of these kinases exerted potent cytotoxic effects specifically on p53-deficient tumor cells [136–139]. These studies thus suggest that at least one functional DNA damage-induced cell cycle checkpoint has to be maintained for cancer cells to survive, thereby making the unaffected checkpoint in cancer cells a prime target for therapeutic intervention.

In addition to the necessity for a functional G2/M cell cycle checkpoint in *TP53* mutant cells, the proper resolution of replication stress seems to be of utmost importance for the survival of these cells. Both ATR and its downstream kinase CHEK1 have been shown to allow *p53*-null cells to survive [140–142]. Indeed, inhibition of CHEK1 was shown to result in further accumulation of DNA damage in addition to that caused by the lack of TP53 [141].

As mentioned earlier, loss of *TP53* is able to induce a switch in glucose metabolism from mitochondrial respiration to glycolysis [143–145]. Interestingly, the dependency of *TP53*-null cells on

glycolysis could be reversed by depletion of PIP4K2. Reduction of the levels of PIP4K2 in TP53-deficient breast carcinoma lines led to severe growth impairment *in vitro*, while deletion of PIP4K2 significantly improved overall survival and reduced the tumor burden in *TP53* knockout mice [146]. These striking effects were likely caused by an increase in the levels of ROS and an impaired glucose metabolism, suggesting that PIP4K2 is needed to sustain the switch in glucose metabolism observed in *TP53*-null cells [146,147].

A rather unexpected synthetic lethal interaction was identified between TP53 and POLR2A. This interaction cannot be linked specifically to the function of TP53, but rather originates from a general mode of p53 deletion. Liu and coworkers observed that in a large fraction of colorectal cancers, a deletion of *TP53* was invariably accompanied by deletion of essential neighboring genes. They went on to show that inhibition of one such gene, POLR2A, could inhibit proliferation and decrease survival in cells that showed hemizygous loss of *TP53* [148]. This finding encourages research on synthetic lethal partners of TP53 to also focus outside the field of cell cycle and DNA damage processes. However, caution has to be taken with an approach such as the one that was taken in the study of Liu *et al*, as dose-dependent side effects are to be expected when this finding is translated to the clinical setting.

RB1

The function of the RB1 tumor suppressor gene is affected by mutations or deletions in a number of specific tumor types. This gene is lost in the large majority of retinoblastomas, small-cell lung cancers, neuroendocrine prostate cancers, and osteosarcomas. Additionally, it is affected in a subset of pituitary, breast, and bladder cancers [149]. The main biological activity of RB1 is its ability to control the cell cycle checkpoint at G1/S transition by binding to E2F family transcription factors and thereby repressing E2F target genes. E2F factors regulate transcription of a multitude of genes involved in cell cycle progression and checkpoint control, nucleotide synthesis, and DNA replication and repair [150]. Once RB1 is lost, the inhibitory capacity of this protein is abrogated, leading to an increase in E2F-mediated transcription and a consequent loss of the G1/S checkpoint.

It is presently impossible to restore the function of RB1 in cancer cells that have lost this gene; therefore, several studies have explored alternative ways to target RB1-deficient tumors. The functional identity of several synthetic lethal partners of RB1 can be explained by the fact that E2F transcription factors can promote both proliferation and apoptosis. As part of a safeguard mechanism, the level of E2F1 is tightly regulated in order to prevent the induction of apoptosis [151,152]. As a direct feedback mechanism resulting from its transcriptional activity, high levels of E2F lead to activation of the cyclin A/CDK2 complex, which in turn can bind and phosphorylate E2F1. This phosphorylation promotes E2F's dissociation from the DNA and inactivates transcription of its target genes, thereby keeping E2F activity in check [153,154]. Blocking the interaction of cyclin A/CDK2 with E2F1 using inhibitory peptides resulted in targeted apoptosis of cells with disrupted RB1 pathway due to the enhanced transcriptional activity of E2F1 [155].

Other studies reported the synthetic lethality between RB1 and SKP2, which possibly shows another mechanism that affects the same feedback loop. SKP2 is an E3 ubiquitin ligase responsible for targeting the cyclin-dependent kinase inhibitor p27 for proteasomal degradation [156]. SKP2 is in turn targeted for degradation by

anaphase-promoting complex/cyclosome (APC/C), and pRB serves as an adaptor protein to bring APC/C and SKP2 together [157]. This regulatory network was confirmed by a study that showed that re-expression of pRB in the *RB1*-deficient Saos-2 osteosarcoma cell line resulted in accumulation of p27, which is an early event occurring before RB1-mediated repression of E2F target genes [158]. Interestingly, deletion of both *Rb1* and *Skp2* resulted in synthetic lethality in the context of pituitary tumor formation in mice [159]. Specifically, while *Rb1*-deficient mice developed pituitary tumors, no tumor formation was observed when both *Rb1* and *Skp2* were deleted [159]. The authors went on to show that this synthetic lethality is caused via the activity of p27, as a non-degradable mutant form of p27 mimicked the effect of *Skp2* deletion [159]. Possibly, the synthetic lethality between *SKP2* and *RB1* could stem from the feedback mechanism described between cyclin A/CDK2 and E2F. As p27 levels rise upon SKP2 inhibition, it would be able to inhibit cyclin A/CDK2 activity, thereby overactivating E2F [160]. Indeed, more recently it was shown that SKP2 is able to limit the activity of E2F1 and thereby suppresses apoptosis in RB1-deficient cells [161].

Another synthetic lethal interaction with RB1 loss was uncovered following a genetic screen carried out in a *Drosophila* mutant for the fly homolog of *RB1 (rbf)*. The screen identified the fly homolog of *TSC2 (gig)* as a synthetic lethal partner of *rbf*. TSC2 is a GTPase-activating protein for RHEB, leading to inactivation of this protein and downregulation of mTOR activity [162,163]. mTOR senses and responds to changes in cellular growth conditions, and its activation by growth factors and mitogens leads to increased protein synthesis. In low-nutrient conditions and cellular stress, TSC2 is activated by phosphorylation and shuts down mTORC1 function through inactivation of RHEB. Conversely, inhibition of TSC2 leads to an increase of protein synthesis and mitochondrial oxidative phosphorylation and a decrease in *de novo* lipid synthesis, which can lead to metabolic, oxidative, and ER stress [164,165].

Inactivation of both *rbf* and *gig* resulted in synergistic induction of cell death in normal developing tissues [166]. Furthermore, inhibition of human TSC2 using shRNAs in a human prostate cancer cell line led to cell death of the RB1-mutant cell line, but not of RB1 wild-type counterpart. The same held true for other RB1-mutant cell lines, such as the osteosarcoma SAOS-2 and breast cancer MDA-MB-468 lines [166]. The likely mechanism was proposed to be an accumulated increase in ROS levels, particularly in hypoxic conditions, which lead to oxidative stress. In addition, inhibition of both *rbf* and *gig* correlated with increased metabolic stress, as well as with defects in G1-S control, as a consequence of DNA damage [167]. These interactions could directly affect processes regulated by RB1 as it has been shown to play an important role in regulation of cell metabolism and stress induction [168–170]. Inhibition of TSC2 could thus be considered as an approach to specifically kill RB1-mutant cancers, but this is complicated by the fact that inhibition of TSC2 in normal tissues can lead to tumor formation [171]. It has been argued, however, that short-term inactivation of TSC2 may provide a therapeutic window.

In a recent study, a novel synthetic lethal interaction was uncovered between RB1 inactivation and a deregulated WNT pathway. Inactivation of the WNT pathway induced synergistic apoptosis with mutation of *RB1* in *Drosophila*. Interestingly, this was also shown to be a consequence of elevated mTOR activity and excessive metabolic stress [172].

Another synthetic lethal interaction was identified between RB1 and MED4, a subunit of the mediator complex that couples specific transcription factors with the RNA polymerase II transcription machinery [173,174]. *RB1* knockout cells could not survive without MED4 both *in vitro* and *in vivo*, suggesting that MED4 is a survival gene in retinoblastoma. This explained an observation that patients with large *RB1* deletions that encompass both *RB1* and *MED4* develop retinoblastoma only sporadically [174]. The function of MED4 is still unknown, and given its ubiquitous expression, it may be that its activity is required for all cell types and is not specific for RB1-negative cells. Further studies eliminating MED4 in distinct cell types should therefore be performed to understand its role in normal physiology and cancer progression.

MYC

MYC, MYCN, and MYCL constitute the MYC family of transcription factors that are among the most frequently activated oncogenes in human cancers. They regulate a variety of oncogenic functions, increase the rate of DNA synthesis, stimulate transcription and ribosome synthesis, regulate RNA processing, and drive expression of metabolic enzymes [175]. As a result, cells that harbor activated MYC genes are characterized by overactive transcription, translation, and metabolism. These characteristics provide cells with faster proliferation rate, the propensity for self-renewal, and independence from growth signals [175]. Furthermore, MYC overexpression has an important role in generating a tumor-supporting microenvironment [176]. On the other hand, this same signaling induces a hypersensitivity to apoptotic stimuli, increased levels of DNA damage and replication stress, and an increased dependency on cell components needed for efficient growth [175]. As MYC is currently not effectively druggable, multiple distinct non-oncogene addictions may provide alternative options to treat MYC-driven cancers.

Intracellular MYC levels are tightly regulated, and overexpression of MYC sensitizes cells to apoptotic stimuli [177,178]. For example, elevated levels of MYC lead to upregulation of DR5, a receptor for TRAIL [179]. It is therefore not surprising that synthetic lethal partners of MYC that impinge on the apoptotic process have been identified. Specifically, inhibition of GSK3B or of FBXW7 potentiated apoptosis of MYC-expressing cells [180,181]. As GSK3B and FBXW7 work in concert to target MYC for degradation, their inhibition leads to stabilization of MYC protein and sensitizes cells to DR5-mediated apoptosis. The GSK3B/FBXW7 axis may therefore be functioning as a buffer to keep MYC at a tolerable level, and prevents activation of the DR5/TRAIL pathway [179,180].

MYC has been shown to induce DNA damage and lead to genomic instability [43], which can result in apoptosis or senescence. To overcome this, tumor cells frequently harbor mutations in components of DNA damage response pathways [182,183]. Due to aberrant sensing and repair of this damage, these cells survive and continue to proliferate in the presence of elevated levels of DNA damage. Even though MYC-dependent tumors seem to allow for DNA damage to reach extensive levels, their stress buffering capacities can be worn out. Various components of DNA damage repair pathways and regulators of replication stress have been identified as synthetic lethal partners of MYC, including PRKDC [82,184]. Inhibition of PRKDC, a kinase with a critical role in NHEJ-mediated DNA repair, induced apoptosis as a consequence of further elevated DNA damage levels [184]. As discussed earlier, oncogene-induced

replication stress also leads to DNA damage and to a dependency on the main regulators of these stress levels, ATR and CHEK1. Therefore, MYC-driven tumors can be selectively killed by inhibition of either of these proteins [114,185,186].

Several studies have now shown that the increase in DNA damage-related stress induced by MYC leads to an enhanced dependency on mitotic checkpoint regulation to allow for proper cell division. MYC-driven tumor cells were shown to depend on the mitotic regulators AURKA, AURKB, and CDK1 for their survival, as inhibition of these proteins resulted in apoptosis and mitotic catastrophe [187–191]. Overall, these studies suggest a critical dependency of MYC-driven tumors on a properly functioning mitotic checkpoint, even though it cannot be excluded that the Aurora kinases could play a direct role in the stabilization of MYC. Similarly, the SUMO-activating enzymes SAE1/2 were shown to be critical factors for proper division of MYC-dependent tumor cells. Inhibition of SAE1 and SAE2 in cells expressing a MYC transgene led to mitotic catastrophe and cell death, suggesting that sumoylation plays a major role in mitotic spindle formation in these cells [192].

As the MYC family members are powerful transcription factors that regulate thousands of genes, their activation could lead to enhanced levels of stress in the mRNA processing machinery. Increased transcription in MYC-driven cells without concomitant upregulation of spliceosome components may lead to intron retention and a reduction of mRNA stability and levels. Not surprisingly, cell lines derived from MYC-dependent cancers showed an increased sensitivity to core spliceosome depletion. Specifically, BUD31, a component of the core spliceosome, was found to be a synthetic lethal partner of MYC. This dependency on the spliceosome may be caused by the addiction of these cells to continuous production of specific oncogenic mRNAs. Alternatively, this phenomenon could be explained by a more general non-oncogene addiction to enhanced levels of mRNA processing machinery induced by MYC-driven transcription [193]. In line with this, MYC-driven tumor cells seem to be highly vulnerable to inhibition of translation. MYC and mTOR were shown to work in concert to induce phosphorylation of EIF4EBP1. This phosphorylation prevents the binding of EIF4EBP1 to EIF4E, thereby activating EIF4E's capacity to stimulate translation initiation [194]. The continuous phosphorylation of EIF4EBP1 by mTOR was shown to be crucial for enhancing translation and prosurvival signaling in MYC-driven tumor cells, and inhibition of mTOR showed high efficacy in MYC-dependent hematological cancers [195,196]. Additionally, in a genomewide loss-of-function screen, many genes functioning in the basic transcription machinery and ribosomal RNA synthesis were identified as synthetic lethal partners of MYC, suggesting that MYC-driven tumors highly depend on the processes of transcription and translation [82].

All the aforementioned studies show that even though it is not currently possible to directly target MYC, there are many alternative routes to selectively target MYC-driven tumors. The broad availability of inhibitors of mitotic checkpoint kinases and motor proteins suggests that targeting mitotic regulation might be the most easily applied means for therapeutic intervention for these tumors.

RAS

The RAS family of oncogenes, which includes KRAS, HRAS, and NRAS, contains the first oncogenes to be described and are found mutated in about 25–30% of human cancers [197]. Normally, RAS

genes are activated by growth factor receptors, after which they relay signals downstream via the MAPK pathway. The constitutive activation of RAS oncogenes, most commonly by point mutations, leads to alterations in many processes such as cell cycle progression, growth, migration, cytoskeletal changes, apoptosis, and senescence [197,198]. Due to dependency of a large fraction of tumors on RAS oncogenes for their survival, a variety of drugs have been developed to specifically target these oncogenic proteins. Initial attempts were focused on blocking farnesylation, a common posttranslational modification of RAS, which showed great promise in preclinical studies [197]. Unfortunately, subsequent clinical benefit of farnesyl transferase inhibitors could not be shown, which leaves RAS difficult to drug although inhibitors targeting specific RAS mutants are in development [199].

More recently, efforts have been made to understand the non-oncogene addictions that are induced by RAS signaling. Consistent with the broad cellular involvement of RAS signaling, the identified synthetic lethal partners of RAS genes also function in multiple distinct pathways. Oncogenic signaling generally leads to an induction of replication stress, a phenotype that has been studied in detail in RAS-mutated cells. Consequently, a number of synthetic lethal partners of RAS play a role in the regulation of replication or in resolving the stresses it delivers. Cyclin A2 and CDC6, both important for the initiation of replication, have been identified as synthetic lethal partners of RAS [39,200]. Even though these genes are directly involved in the replication process, it cannot be excluded that they serve as factors necessary to sustain the continuous cell cycling induced by RAS instead of being involved in buffering the elevated level of replication stress. ATR and CHEK1 are key proteins that respond to replication stress and initiate the repair of damage induced by the activation of RAS, and RAS-mutated cells are highly sensitive to the inhibition of either of these genes [201,202].

Likely due to their high proliferative capacity and increased DNA damage, RAS-mutated cells suffer from elevated levels of mitotic stress and are highly susceptible to interference with the mitotic checkpoint. In several unbiased screens for synthetic lethal partners of RAS, the mitotic regulators APC/C, PLK1, and KIF2C were identified among the strongest hits [39].

Somewhat surprisingly, activation of individual kinases that can directly induce RAS or that relay its signal was shown to exert a synthetic lethal effect in the presence of a mutated RAS gene. In a recent study on 600 lung adenocarcinoma cases, EGFR and RAS mutations were found to be mutually exclusive. The long-standing dogma implied that because these two genes operate in the same signaling pathway, activation of both would not occur as it poses no selective advantage [203]. Unni and coworkers, however, showed that activation of EGFR in mutant RAS-expressing cells decreased viability, and is highly disadvantageous for tumor cell survival. When tumors were induced in mice by the activation of both of these oncogenes, only tumors that express one of the oncogenes could develop [204]. Along the same lines, activation of BRAF in NRAS-mutated cells led to an induction of the senescent program, even though BRAF is a kinase directly downstream of RAS [205]. These results indicate that a fine balance in the level of oncogenic signaling has to be maintained and that oncogenic overdosing can also be detrimental to tumor cells.

RAS-mutated cells were also reported to depend on several transcription factors for their survival. In particular, the expression of GATA2 and WT1 is crucial for the survival of RAS-driven cells [200,206]. Not only WT1 itself but also one of its targets, SNAI2, has been reported to be a synthetic lethal partner for RAS [207]. It was proposed that RAS-mutated cells rely on WT1 signaling to induce SNAI2 in order to maintain an EMT phenotype. The dependency of RAS-mutated cells on GATA2 requires the regulation of the NFκB pathway and the proteasome [208]. Components of these highly connected pathways were already identified as synthetic lethal partners of RAS, suggesting that the reduction of proteotoxic stress is of critical importance for cells harboring an activated RAS gene [39,209].

Knocking down individual interphase CDKs blocked proliferation of NSCLC cell lines with mutant *KRAS*, while not affecting the growth of cell lines with wild-type *KRAS* allele. Ablation of *Cdk4* in the presence of a mutant *Kras* allele *in vivo* hindered the development of lung tumors. Importantly, loss of *Cdk4* prevented development of aggressive lung adenocarcinomas through induction of senescence, which was accompanied by an increased DNA damage response [210]. Nanoparticles containing siRNAs targeting *CDK4* decreased proliferation of KRAS mutant NSCLC cells but not the KRAS wild-type cells, while their systemic administration inhibited tumor growth in a xenograft murine model [211].

Concluding remarks

With the results of many genomewide genetic screening efforts, our understanding of pathway interactions and the functional consequences of their signaling has been greatly enhanced. In particular, the identification of many synthetic lethal interactions and non-oncogene addictions has opened up novel routes for targeting cancer. Many of these findings do, however, still await further

Table 1. Overview of the described synthetic lethal partners and the non-oncogene addictions they are associated with.

	Proteotoxic stress	Oxidative stress	Metabolic stress	Mitotic stress	DNA damage/replication stress	Non-stress-related
PTEN	mTOR		mTOR	ATM, NLK, PLK4, MPS1	ATM, PARP, PNKP	
TP53		PIP4K2	PIP4K2	AURKA, CDK1, PLK1, WEE1	PLK1, WEE1, ATR, CHEK1	POLR2A
RB1	SKP2, CCNA, CDK2, TSC2	TSC2	TSC2, WNT			
MYC	mTOR, BUD31, EIF4EBP, GSK3B, FBXW7		mTOR	AURKA, AURKB, CDK1, SAE1, SAE2	PRKDC, ATR, CHK1	
RAS	GATA2, TBK1			APC/C, PLK1, KIF2C	Cyclin A2, CDC6, ATR, CHEK1	EGFR, BRAF, WT1, SNAI2, CDK4

clinical validation, which needs to show significant patient benefit. In cases where these synthetic lethal interactions have been explored in patients, the results were often disappointing. Whether this is due to the far more extensive heterogeneity of tumor cells *in vivo*, including their variable microenvironment and associated signaling, as compared to the highly homogeneous cell culture conditions in which screens are generally performed, remains an open question. It emphasizes the importance for careful *in vivo* validation in realistic tumor models (Box 1).

As appearance of resistant clones is almost invariably observed when oncogene addictions are targeted, the targeting of non-oncogene addictions by single inhibitors would also be expected to result in the generation of escape mechanisms. This phenomenon is tightly linked to the genetic instability observed in tumor cells, which allows the cells to acquire genetic lesions that confer resistance to drug treatment. It is to be expected, however, that targeting of a combination of different non-oncogene addictions or combinations of oncogene and non-oncogene additions would limit the possibilities of escape and thereby provide a clinical benefit. Future work should therefore be dedicated toward the identification of possible resistance mechanisms and the putative combinatorial treatments that include targeting of non-oncogene addictions.

Overall, the work discussed in this review indicates that in contrast to non-transformed cells, tumor cells suffer from multiple forms of stress. In general, many of the identified synthetic lethal

interactions indicate that cancer cells heavily rely on several pathways that are able to relieve these stresses for their survival. With regard to therapeutic opportunities, this points to the notion that tipping the balance toward an overkill of stress remains a promising avenue to induce tumor cell death. In most instances, the synthetic lethal interactions can be linked to specific antistress defense mechanisms, and therefore can be considered a subclass of non-oncogene addictions (Table 1). Strikingly, many of the synthetic lethal interactions affect processes that are often targeted by classical chemotherapeutics, namely DNA damage and mitotic stress. Recent work on synthetic lethality suggests that application of more DNA damage or mitotic-related stress remains a potent approach to kill tumor cells. It also suggests that other stresses, such as proteotoxic and metabolic stress, which are usually elevated in tumor cells, could be exploited for therapeutic intervention. With the advent of more targeted agents that can specifically impinge on these processes, one expects that this should reduce side effects, while generating similar detrimental stress levels specifically in cancer cells.

Acknowledgements

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, Allen C, Hansen G, Woodbrook R, Wolfe C *et al* (2015) The Global Burden of Cancer 2013. *JAMA Oncol* 1: 505–527

2. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144: 646–674

3. Galluzzi L, Vitale I, Michels J, Brenner C, Szabadkai G, Harel-Bellan A, Castedo M, Kroemer G (2014) Systems biology of cisplatin resistance: past, present and future. *Cell Death Dis* 5: e1257

4. Risinger AL, Mooberry SL (2010) Taccalonolides: novel microtubule stabilizers with clinical potential. *Cancer Lett* 291: 14–19

5. Kaelin WG (2005) The concept of synthetic lethality in the context of anticancer therapy. *Nat Rev Cancer* 5: 689–698

6. Semenova EA, Nagel R, Berns A (2015) Origins, genetic landscape, and emerging therapies of small cell lung cancer. *Genes Dev* 29: 1447–1462

7. Nagel R, Martens-de Kemp SR, Buijze M, Jacobs G, Braakhuis BJ, Brakenhoff RH (2013) Treatment response of HPV-positive and HPV-negative head and neck squamous cell carcinoma cell lines. *Oral Oncol* 49: 560–566

8. Dasari S, Tchounwou PB (2014) Cisplatin in cancer therapy: molecular mechanisms of action. *Eur J Pharmacol* 740: 364–378

9. Ferrari E, Lucca C, Foiani M (2010) A lethal combination for cancer cells: synthetic lethality screenings for drug discovery. *Eur J Cancer* 46: 2889–2895

10. Weinstein IB (2002) Cancer. Addiction to oncogenes–the Achilles heal of cancer. *Science* 297: 63–64

11. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M *et al* (2001) Use of

chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 344: 783–792

12. Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley ML, Gathmann I, Bolton AE, van Hoomissen IC, Goldman JM *et al* (2003) Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med* 349: 1423–1432

13. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen C-J, Van den Abbeele AD, Druker BJ *et al* (2003) Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* 21: 4342–4349

14. Kwak EL, Bang Y-J, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou S-HI, Dezube BJ, Jänne PA, Costa DB *et al* (2010) Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 363: 1693–1703

15. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG *et al* (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 350: 2129–2139

16. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K *et al* (2010) Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 363: 809–819

17. Felsher DW, Bishop JM (1999) Reversible tumorigenesis by MYC in hematopoietic lineages. *Mol Cell* 4: 199–207

18. Fisher GH, Wellen SL, Klimstra D, Lenczowski JM, Tichelaar JW, Lizak MJ, Whitsett JA, Koretsky A, Varmus HE (2001) Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes. *Genes Dev* 15: 3249–3262

19. Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH (1997) Integrating genetic approaches into the discovery of anticancer drugs. *Science* 278: 1064–1068

20. Lucchesi JC (1968) Synthetic lethality and semi-lethality among functionally related mutants of *Drosophila melanogaster*. *Genetics* 59: 37–44

21. Luo J, Solimini NL, Elledge SJ (2009) Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell* 136: 823–837

22. Freije JMP, Fraile JM, López-Otín C (2011) Protease addiction and synthetic lethality in cancer. *Front Oncol* 1: 25

23. Solimini NL, Luo J, Elledge SJ (2007) Non-oncogene addiction and the stress phenotype of cancer cells. *Cell* 130: 986–988

24. Marcotte R, Brown KR, Suarez F, Sayad A, Karamboulas K, Krzyzanowski PM, Sircoulomb F, Medrano M, Fedyshyn Y, Koh JLY *et al* (2012) Essential gene profiles in breast, pancreatic, and ovarian cancer cells. *Cancer Discov* 2: 172–189

25. Martens-de Kemp SR, Nagel R, Stigter-van Walsum M, van der Meulen IH, van Beusechem VW, Braakhuis BJ, Brakenhoff RH (2013) Functional genetic screens identify genes essential for tumor cell survival in head and neck and lung cancer. *Clin Cancer Res* 19: 1994–2003

26. Nagel R, Stigter-van Walsum M, Buijze M, van den Berg J, van der Meulen IH, Hodzic J, Piersma SR, Pham TV, Jimenez CR, van Beusechem VW *et al* (2015) Genome-wide siRNA screen identifies the radiosensitizing effect of downregulation of MASTL and FOXM1 in NSCLC. *Mol Cancer Ther* 14: 1434–1444

27. Jolly C, Morimoto RI (2000) Role of the heat shock response and molecular chaperones in oncogenesis and cell death. *J Natl Cancer Inst* 92: 1564–1572

28. Whitesell L, Lindquist SL (2005) HSP90 and the chaperoning of cancer. *Nat Rev Cancer* 5: 761–772

29. Dai C, Whitesell L, Rogers AB, Lindquist S (2007) Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. *Cell* 130: 1005–1018

30. Xiao X, Zuo X, Davis AA, McMillan DR, Curry BB, Richardson JA, Benjamin IJ (1999) HSF1 is required for extra-embryonic development, postnatal growth and protection during inflammatory responses in mice. *EMBO J* 18: 5943–5952

31. Torres EM, Sokolsky T, Tucker CM, Chan LY, Boselli M, Dunham MJ, Amon A (2007) Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science* 317: 916–924

32. Oromendia AB, Dodgson SE, Amon A (2012) Aneuploidy causes proteotoxic stress in yeast. *Genes Dev* 26: 2696–2708

33. Santaguida S, Vasile E, White E, Amon A (2015) Aneuploidy-induced cellular stresses limit autophagic degradation. *Genes Dev* 29: 2010–2021

34. Shor E, Fox CA, Broach JR (2013) The yeast environmental stress response regulates mutagenesis induced by proteotoxic stress. *PLoS Genet* 9: e1003680

35. He X, Arrotta N, Radhakrishnan D, Wang Y, Romigh T, Eng C (2013) Cowden syndrome-related mutations in PTEN associate with enhanced proteasome activity. *Cancer Res* 73: 3029–3040

36. Glick D, Barth S, Macleod KF (2010) Autophagy: cellular and molecular mechanisms. *J Pathol* 221: 3–12

37. Ozpolat B, Benbrook DM (2015) Targeting autophagy in cancer management – strategies and developments. *Cancer Manage Res* 7: 291–299

38. Leu JI-J, Pimkina J, Frank A, Murphy ME, George DL (2009) A small molecule inhibitor of inducible heat shock protein 70. *Mol Cell* 36: 15–27

39. Luo J, Emanuele MJ, Li D, Creighton CJ, Schlabach MR, Westbrook TF, Wong K-K, Elledge SJ (2009) A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. *Cell* 137: 835–848

40. Neckers L, Workman P (2012) Hsp90 molecular chaperone inhibitors: are we there yet? *Clin Cancer Res* 18: 64–76

41. Tong L, Chuang CC, Wu S, Zuo L (2015) Reactive oxygen species in redox cancer therapy. *Cancer Lett* 367: 18–25

42. Lee AC, Fenster BE, Ito H, Takeda K, Bae NS, Hirai T, Yu ZX, Ferrans VJ, Howard BH, Finkel T (1999) Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. *J Biol Chem* 274: 7936–7940

43. Vafa O, Wade M, Kern S, Beeche M, Pandita TK, Hampton GM, Wahl GM (2002) c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. *Mol Cell* 9: 1031–1044

44. Luo M, He H, Kelley MR, Georgiadis MM (2010) Redox regulation of DNA repair: implications for human health and cancer therapeutic development. *Antioxid Redox Signal* 12: 1247–1269

45. Huber KVM, Salah E, Radic B, Gridling M, Elkins JM, Stukalov A, Jemth A-S, Göktürk C, Sanjiv K, Strömberg K *et al* (2014) Stereospecific targeting of MTH1 by (S)-crizotinib as an anticancer strategy. *Nature* 508: 222–227

46. Gad H, Koolmeister T, Jemth A-S, Eshtad S, Jacques SA, Ström CE, Svensson LM, Schultz N, Lundbäck T, Einarsdottir BO *et al* (2014) MTH1 inhibition eradicates cancer by preventing sanitation of the dNTP pool. *Nature* 508: 215–221

47. Malhotra D, Portales-Casamar E, Singh A, Srivastava S, Arenillas D, Happel C, Shyr C, Wakabayashi N, Kensler TW, Wasserman WW *et al*

(2010) Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. *Nucleic Acids Res* 38: 5718–5734

48. Zhu H, Itoh K, Yamamoto M, Zweier JL, Li Y (2005) Role of Nrf2 signaling in regulation of antioxidants and phase 2 enzymes in cardiac fibroblasts: protection against reactive oxygen and nitrogen species-induced cell injury. *FEBS Lett* 579: 3029–3036

49. Xu C, Huang M-T, Shen G, Yuan X, Lin W, Khor TO, Conney AH, Kong A-NT (2006) Inhibition of 7,12-dimethylbenz(a)anthracene-induced skin tumorigenesis in C57BL/6 mice by sulforaphane is mediated by nuclear factor E2-related factor 2. *Cancer Res* 66: 8293–8296

50. Ramos-Gomez M, Dolan PM, Itoh K, Yamamoto M, Kensler TW (2003) Interactive effects of nrf2 genotype and oltipraz on benzo[a]pyrene-DNA adducts and tumor yield in mice. *Carcinogenesis* 24: 461–467

51. Padmanabhan B, Tong KI, Ohta T, Nakamura Y, Scharlock M, Ohtsuji M, Kang M-I, Kobayashi A, Yokoyama S, Yamamoto M (2006) Structural basis of defects of Keap1 activity provoked by its point mutations in lung cancer. *Mol Cell* 21: 689–700

52. Ohta T, Iijima K, Miyamoto M, Nakahara I, Tanaka H, Ohtsuji M, Suzuki T, Kobayashi A, Yokota J, Sakiyama T *et al* (2008) Loss of Keap1 function activates Nrf2 and provides advantages for lung cancer cell growth. *Cancer Res* 68: 1303–1309

53. Shibata T, Ohta T, Tong KI, Kokubu A, Odogawa R, Tsuta K, Asamura H, Yamamoto M, Hirohashi S (2008) Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. *Proc Natl Acad Sci USA* 105: 13568–13573

54. Solis LM, Behrens C, Dong W, Suraokar M, Ozburn NC, Moran CA, Corvalan AH, Biswal S, Swisher SG, Bekele BN *et al* (2010) Nrf2 and Keap1 abnormalities in non-small cell lung carcinoma and association with clinicopathologic features. *Clin Cancer Res* 16: 3743–3753

55. Satoh H, Moriguchi T, Takai J, Ebina M, Yamamoto M (2013) Nrf2 prevents initiation but accelerates progression through the Kras signaling pathway during lung carcinogenesis. *Cancer Res* 73: 4158–4168

56. Raj L, Ide T, Gurkar AU, Foley M, Schenone M, Li X, Tolliday NJ, Golub TR, Carr SA, Shamji AF *et al* (2011) Selective killing of cancer cells by a small molecule targeting the stress response to ROS. *Nature* 475: 231–234

57. Trachootham D, Zhou Y, Zhang H, Demizu Y, Chen Z, Pelicano H, Chiao PJ, Achanta G, Arlinghaus RB, Liu J *et al* (2006) Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by β-phenylethyl isothiocyanate. *Cancer Cell* 10: 241–252

58. Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Bonnet S *et al* (2007) A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. *Cancer Cell* 11: 37–51

59. Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324: 1029–1033

60. Campbell PN (1993) Principles of biochemistry second edition. *Biochem Educ* 21: 114

61. Mazurek S, Boschek CB, Hugo F, Eigenbrodt E (2005) Pyruvate kinase type M2 and its role in tumor growth and spreading. *Semin Cancer Biol* 15: 300–308

62. Erez A, DeBerardinis RJ (2015) Metabolic dysregulation in monogenic disorders and cancer – finding method in madness. *Nat Rev Cancer* 15: 440–448

63. Chiaradonna F, Gaglio D, Vanoni M, Alberghina L (2006) Expression of transforming K-Ras oncogene affects mitochondrial function and morphology in mouse fibroblasts. *Biochim Biophys Acta* 1757: 1338–1356

64. Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR, Zhuang H, Cinalli RM, Alavi A, Rudin CM *et al* (2004) Akt stimulates aerobic glycolysis in cancer cells. *Cancer Res* 64: 3892–3899

65. Matoba S, Kang J-G, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurley PJ, Bunz F, Hwang PM (2006) p53 regulates mitochondrial respiration. *Science* 312: 1650–1653

66. Buzzai M, Bauer DE, Jones RG, Deberardinis RJ, Hatzivassiliou G, Elstrom RL, Thompson CB (2005) The glucose dependence of Akt-transformed cells can be reversed by pharmacologic activation of fatty acid beta-oxidation. *Oncogene* 24: 4165–4173

67. Son J, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M, Perera RM, Ferrone CR, Mullarky E, Shyh-Chang N *et al* (2013) Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* 496: 101–105

68. Iurlaro R, León-Annicchiarico CL, Muñoz-Pinedo C (2014) Regulation of cancer metabolism by oncogenes and tumor suppressors. *Methods Enzymol* 542: 59–80

69. Wu M, Neilson A, Swift AL, Moran R, Tamagnine J, Parslow D, Armistead S, Lemire K, Orrell J, Teich J *et al* (2007) Multiparameter metabolic analysis reveals a close link between attenuated mitochondrial bioenergetic function and enhanced glycolysis dependency in human tumor cells. *Am J Physiol Cell Physiol* 292: C125–C136

70. Halicka HD, Ardelt B, Li X, Melamed MM, Darzynkiewicz Z (1995) 2-Deoxy-D-glucose enhances sensitivity of human histiocytic lymphoma U937 cells to apoptosis induced by tumor necrosis factor. *Cancer Res* 55: 444–449

71. Geschwind J-FH, Ko YH, Torbenson MS, Magee C, Pedersen PL (2002) Novel therapy for liver cancer: direct intraarterial injection of a potent inhibitor of ATP production. *Cancer Res* 62: 3909–3913

72. Jaroszewski JW, Kaplan O, Cohen JS (1990) Action of gossypol and rhodamine 123 on wild type and multidrug-resistant MCF-7 human breast cancer cells: 31P nuclear magnetic resonance and toxicity studies. *Cancer Res* 50: 6936–6943

73. Kaplon J, Zheng L, Meissl K, Chaneton B, Selivanov VA, Mackay G, van der Burg SH, Verdegaal EME, Cascante M, Shlomi T *et al* (2013) A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. *Nature* 498: 109–112

74. Li J, Zhu J, Melvin WS, Bekaii-Saab TS, Chen C-S, Muscarella P (2006) A structurally optimized celecoxib derivative inhibits human pancreatic cancer cell growth. *J Gastrointest Surg* 10: 207–214

75. McFate T, Mohyeldin A, Lu H, Thakar J, Henriques J, Halim ND, Wu H, Schell MJ, Tsang TM, Teahan O *et al* (2008) Pyruvate dehydrogenase complex activity controls metabolic and malignant phenotype in cancer cells. *J Biol Chem* 283: 22700–22708

76. Jain M, Nilsson R, Sharma S, Madhusudhan N, Kitami T, Souza AL, Kafri R, Kirschner MW, Clish CB, Mootha VK (2012) Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. *Science* 336: 1040–1044

77. Maddocks ODK, Berkers CR, Mason SM, Zheng L, Blyth K, Gottlieb E, Vousden KH (2013) Serine starvation induces stress and p53-dependent metabolic remodelling in cancer cells. *Nature* 493: 542–546

78. Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K, Sethumadhavan S, Woo H-K, Jang HG, Jha AK *et al* (2011) Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 476: 346–350

79. Scott L, Lamb J, Smith S, Wheatley DN (2000) Single amino acid (arginine) deprivation: rapid and selective death of cultured transformed and malignant cells. *Br J Cancer* 83: 800–810

80. Yuneva M, Zamboni N, Oefner P, Sachidanandam R, Lazebnik Y (2007) Deficiency in glutamine but not glucose induces MYC-dependent apoptosis in human cells. *J Cell Biol* 178: 93–105

81. Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang X-Y, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB *et al* (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc Natl Acad Sci USA* 105: 18782–18787

82. Toyoshima M, Howie HL, Imakura M, Walsh RM, Annis JE, Chang AN, Frazier J, Chau BN, Loboda A, Linsley PS *et al* (2012) Functional genomics identifies therapeutic targets for MYC-driven cancer. *Proc Natl Acad Sci USA* 109: 9545–9550

83. Commisso C, Davidson SM, Soydaner-Azeloglu RG, Parker SJ, Kamphorst JJ, Hackett S, Grabocka E, Nofal M, Drebin JA, Thompson CB *et al* (2013) Macropinocytosis of protein is an amino acid supply route in Ras-transformed cells. *Nature* 497: 633–637

84. Sheen J-H, Zoncu R, Kim D, Sabatini DM (2011) Defective regulation of autophagy upon leucine deprivation reveals a targetable liability of human melanoma cells *in vitro* and *in vivo*. *Cancer Cell* 19: 613–628

85. Wilson PM, Danenberg PV, Johnston PG, Lenz H-J, Ladner RD (2014) Standing the test of time: targeting thymidylate biosynthesis in cancer therapy. *Nat Rev Clin Oncol* 11: 282–298

86. Ronghe M, Burke GA, Lowis SP, Estlin EJ (2001) Remission induction therapy for childhood acute lymphoblastic leukaemia: clinical and cellular pharmacology of vincristine, corticosteroids, L-asparaginase and anthracyclines. *Cancer Treat Rev* 27: 327–337

87. Ding Y, Li Z, Broome JD (2005) Epigenetic changes in the repression and induction of asparagine synthetase in human leukemic cell lines. *Leukemia* 19: 420–426

88. Zyromski NJ, Mathur A, Pitt HA, Wade TE, Wang S, Nakshatri P, Swartz-Basile DA, Nakshatri H (2009) Obesity potentiates the growth and dissemination of pancreatic cancer. *Surgery* 146: 258–263

89. Wang F, Herrington M, Larsson J, Permert J (2003) The relationship between diabetes and pancreatic cancer. *Mol Cancer* 2: 4

90. Lee C, Raffaghello L, Brandhorst S, Safdie FM, Bianchi G, Martin-Montalvo A, Pistoia V, Wei M, Hwang S, Merlino A *et al* (2012) Fasting cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy. *Sci Transl Med* 4: 124ra127

91. Birsoy K, Possemato R, Lorbeer FK, Bayraktar EC, Thiru P, Yucel B, Wang T, Chen WW, Clish CB, Sabatini DM (2014) Metabolic determinants of cancer cell sensitivity to glucose limitation and biguanides. *Nature* 508: 108–112

92. Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, Viollet B, Thompson CB (2007) Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res* 67: 6745–6752

93. Delattre M, Gönczy P (2004) The arithmetic of centrosome biogenesis. *J Cell Sci* 117: 1619–1630

94. Chan JY (2011) A clinical overview of centrosome amplification in human cancers. *Int J Biol Sci* 7: 1122–1144

95. Doxsey S (2002) Duplicating dangerously: linking centrosome duplication and aneuploidy. *Mol Cell* 10: 439–440

96. Fukasawa K, Choi T, Kuriyama R, Rulong S, Vande Woude GF (1996) Abnormal centrosome amplification in the absence of p53. *Science* 271: 1744–1747

97. Korzeniewski N, Treat B, Duensing S (2011) The HPV-16 E7 oncoprotein induces centriole multiplication through deregulation of Polo-like kinase 4 expression. *Mol Cancer* 10: 61

98. Starita LM, Machida Y, Sankaran S, Elias JE, Griffin K, Schlegel BP, Gygi SP, Parvin JD (2004) BRCA1-dependent ubiquitination of gamma-tubulin regulates centrosome number. *Mol Cell Biol* 24: 8457–8466

99. Balsitis SJ, Sage J, Duensing S, Münger K, Jacks T, Lambert PF (2003) Recapitulation of the effects of the human papillomavirus type 16 E7 oncogene on mouse epithelium by somatic Rb deletion and detection of pRb-independent effects of E7 *in vivo*. *Mol Cell Biol* 23: 9094–9103

100. Tutt A, Gabriel A, Bertwistle D, Connor F, Paterson H, Peacock J, Ross G, Ashworth A (1999) Absence of Brca2 causes genome instability by chromosome breakage and loss associated with centrosome amplification. *Curr Biol* 9: 1107–1110

101. Galimberti F, Thompson SL, Ravi S, Compton DA, Dmitrovsky E (2011) Anaphase catastrophe is a target for cancer therapy. *Clin Cancer Res* 17: 1218–1222

102. Ring D, Hubble R, Kirschner M (1982) Mitosis in a cell with multiple centrioles. *J Cell Biol* 94: 549–556

103. Quintyne NJ, Reing JE, Hoffelder DR, Gollin SM, Saunders WS (2005) Spindle multipolarity is prevented by centrosomal clustering. *Science* 307: 127–129

104. Kwon M, Godinho SA, Chandhok NS, Ganem NJ, Azioune A, Thery M, Pellman D (2008) Mechanisms to suppress multipolar divisions in cancer cells with extra centrosomes. *Genes Dev* 22: 2189–2203

105. Leber B, Maier B, Fuchs F, Chi J, Riffel P, Anderhub S, Wagner L, Ho AD, Salisbury JL, Boutros M *et al* (2010) Proteins required for centrosome clustering in cancer cells. *Sci Transl Med* 2: 33ra38

106. Watts CA, Richards FM, Bender A, Bond PJ, Korb O, Kern O, Riddick M, Owen P, Myers RM, Raff J *et al* (2013) Design, synthesis, and biological evaluation of an allosteric inhibitor of HSET that targets cancer cells with supernumerary centrosomes. *Chem Biol* 20: 1399–1410

107. Castiel A, Visochek L, Mittelman L, Dantzer F, Izraeli S, Cohen-Armon M (2011) A phenanthrene derived PARP inhibitor is an extra-centrosomes de-clustering agent exclusively eradicating human cancer cells. *BMC Cancer* 11: 412

108. Raab MS, Breitkreutz I, Anderhub S, Rønnest MH, Leber B, Larsen TO, Weiz L, Konotop G, Hayden PJ, Podar K *et al* (2012) GF-15, a novel inhibitor of centrosomal clustering, suppresses tumor cell growth *in vitro* and *in vivo*. *Cancer Res* 72: 5374–5385

109. Kawamura E, Fielding AB, Kannan N, Balgi A, Eaves CJ, Roberge M, Dedhar S (2013) Identification of novel small molecule inhibitors of centrosome clustering in cancer cells. *Oncotarget* 4: 1763–1776

110. Pannu V, Rida PCG, Celik B, Turaga RC, Ogden A, Cantuaria G, Gopalakrishnan J, Aneja R (2014) Centrosome-declustering drugs mediate a two-pronged attack on interphase and mitosis in supercentrosomal cancer cells. *Cell Death Dis* 5: e1538

111. Bartkova J, Horejsí Z, Koed K, Krämer A, Tort F, Zieger K, Guldberg P, Sehested M, Nesland JM, Lukas C *et al* (2005) DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature* 434: 864–870

112. D'Andrea AD, Grompe M (2003) The Fanconi anaemia/BRCA pathway. *Nat Rev Cancer* 3: 23–34

113. Dominguez-Sola D, Ying CY, Grandori C, Ruggiero L, Chen B, Li M, Galloway DA, Gu W, Gautier J, Dalla-Favera R (2007) Non-transcriptional control of DNA replication by c-Myc. *Nature* 448: 445–451

114. Murga M, Campaner S, Lopez-Contreras AJ, Toledo LI, Soria R, Montaña MF, D'Artista L, Schleker T, Guerra C, Garcia E *et al* (2011) Exploiting

oncogene-induced replicative stress for the selective killing of Myc-driven tumors. *Nat Struct Mol Biol* 18: 1331–1335

115. Schoppy DW, Ragland RL, Gilad O, Shastri N, Peters AA, Murga M, Fernandez-Capetillo O, Diehl JA, Brown EJ (2012) Oncogenic stress sensitizes murine cancers to hypomorphic suppression of ATR. *J Clin Invest* 122: 241–252

116. Campaner S, Amati B (2012) Two sides of the Myc-induced DNA damage response: from tumor suppression to tumor maintenance. *Cell Div* 7: 6

117. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD (2015) Homologous recombination deficiency: exploiting the fundamental vulnerability of ovarian cancer. *Cancer Discov* 5: 1137–1154

118. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C *et al* (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 434: 917–921

119. Haince JF, Rouleau M, Hendzel MJ, Masson JY, Poirier GG (2005) Targeting poly(ADP-ribosyl)ation: a promising approach in cancer therapy. *Trends Mol Med* 11: 456–463

120. Ali IU, Schriml LM, Dean M (1999) Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. *J Natl Cancer Inst* 91: 1922–1932

121. Keniry M, Parsons R (2008) The role of PTEN signaling perturbations in cancer and in targeted therapy. *Oncogene* 27: 5477–5485

122. Shen WH, Balajee AS, Wang J, Wu H, Eng C, Pandolfi PP, Yin Y (2007) Essential role for nuclear PTEN in maintaining chromosomal integrity. *Cell* 128: 157–170

123. Mendes-Pereira AM, Martin SA, Brough R, McCarthy A, Taylor JR, Kim J-S, Waldman T, Lord CJ, Ashworth A (2009) Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors. *EMBO Mol Med* 1: 315–322

124. Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, Petersen R, Frost P, Gibbons JJ, Wu H, Sawyers CL (2001) Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc Natl Acad Sci USA* 98: 10314–10319

125. Podsypanina K, Lee RT, Politis C, Hennessy I, Crane A, Puc J, Neshat M, Wang H, Yang L, Gibbons J *et al* (2001) An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten+/- mice. *Proc Natl Acad Sci USA* 98: 10320–10325

126. McEllin B, Camacho CV, Mukherjee B, Hahm B, Tomimatsu N, Bachoo RM, Burma S (2010) PTEN loss compromises homologous recombination repair in astrocytes: implications for glioblastoma therapy with temozolomide or poly(ADP-ribose) polymerase inhibitors. *Cancer Res* 70: 5457–5464

127. Forster MD, Dedes KJ, Sandhu S, Frentzas S, Kristeleit R, Ashworth A, Poole CJ, Weigelt B, Kaye SB, Molife LR (2011) Treatment with olaparib in a patient with PTEN-deficient endometrioid endometrial cancer. *Nat Rev Clin Oncol* 8: 302–306

128. Mereniuk TR, El Gendy MA, Mendes-Pereira AM, Lord CJ, Ghosh S, Foley E, Ashworth A, Weinfeld M (2013) Synthetic lethal targeting of PTEN-deficient cancer cells using selective disruption of polynucleotide kinase/phosphatase. *Mol Cancer Ther* 12: 2135–2144

129. Weinfeld M, Mani RS, Abdou I, Aceytuno RD, Glover JNM (2011) Tidying up loose ends: the role of polynucleotide kinase/phosphatase in DNA strand break repair. *Trends Biochem Sci* 36: 262–271

130. Mendes-Pereira AM, Lord CJ, Ashworth A (2012) NLK is a novel therapeutic target for PTEN deficient tumour cells. *PLoS ONE* 7: e47249

131. Brough R, Frankum JR, Sims D, Mackay A, Mendes-Pereira AM, Bajrami I, Costa-Cabral S, Rafiq R, Ahmad AS, Cerone MA *et al* (2011)

Functional viability profiles of breast cancer. *Cancer Discov* 1: 260–273

132. McCabe N, Hanna C, Walker SM, Gonda D, Li J, Wikstrom K, Savage KI, Butterworth KT, Chen C, Harkin DP *et al* (2015) Mechanistic rationale to target PTEN-deficient tumor cells with inhibitors of the DNA damage response kinase ATM. *Cancer Res* 75: 2159–2165

133. Vogelstein B, Lane D, Levine AJ (2000) Surfing the p53 network. *Nature* 408: 307–310

134. Petitjean A, Mathe E, Kato S, Ishioka C, Tavtigian SV, Hainaut P, Olivier M (2007) Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum Mutat* 28: 622–629

135. Lane DP (1992) Cancer. p53, guardian of the genome. *Nature* 358: 15–16

136. Mizuarai S, Yamanaka K, Itadani H, Arai T, Nishibata T, Hirai H, Kotani H (2009) Discovery of gene expression-based pharmacodynamic biomarker for a p53 context-specific anti-tumor drug Wee1 inhibitor. *Mol Cancer* 8: 34

137. Wang X, Simon R (2013) Identification of potential synthetic lethal genes to p53 using a computational biology approach. *BMC Med Genomics* 6: 30

138. Pappano WN, Zhang Q, Tucker LA, Tse C, Wang J (2014) Genetic inhibition of the atypical kinase Wee1 selectively drives apoptosis of p53 inactive tumor cells. *BMC Cancer* 14: 430

139. Sur S, Pagliarini R, Bunz F, Rago C, Diaz LA, Kinzler KW, Vogelstein B, Papadopoulos N (2009) A panel of isogenic human cancer cells suggests a therapeutic approach for cancers with inactivated p53. *Proc Natl Acad Sci USA* 106: 3964–3969

140. Ruzankina Y, Schoppy DW, Asare A, Clark CE, Vonderheide RH, Brown EJ (2009) Tissue regenerative delays and synthetic lethality in adult mice after combined deletion of Atr and Trp53. *Nat Genet* 41: 1144–1149

141. Ma CX, Cai S, Li S, Ryan CE, Guo Z, Schaiff WT, Lin L, Hoog J, Goiffon RJ, Prat A *et al* (2012) Targeting Chk1 in p53-deficient triple-negative breast cancer is therapeutically beneficial in human-in-mouse tumor models. *J Clin Invest* 122: 1541–1552

142. Kwok M, Davies N, Agathanggelou A, Smith E, Petermann E, Yates E, Brown J, Lau A, Stankovic T (2015) Synthetic lethality in chronic lymphocytic leukaemia with DNA damage response defects by targeting the ATR pathway. *Lancet* 385(Suppl 1): S58

143. Bensaad K, Cheung EC, Vousden KH (2009) Modulation of intracellular ROS levels by TIGAR controls autophagy. *EMBO J* 28: 3015–3026

144. Zhang C, Liu J, Liang Y, Wu R, Zhao Y, Hong X, Lin M, Yu H, Liu L, Levine AJ *et al* (2013) Tumour-associated mutant p53 drives the Warburg effect. *Nat Commun* 4: 2935

145. Kawauchi K, Araki K, Tobiume K, Tanaka N (2008) p53 regulates glucose metabolism through an IKK-NF-kappaB pathway and inhibits cell transformation. *Nat Cell Biol* 10: 611–618

146. Emerling Brooke M, Hurov Jonathan B, Poulogiannis G, Tsukazawa Kazumi S, Choo-Wing R, Wulf Gerburg M, Bell Eric L, Shim H-S, Lamia Katja A, Rameh Lucia E *et al* (2013) Depletion of a putatively druggable class of phosphatidylinositol kinases inhibits growth of p53-null tumors. *Cell* 155: 844–857

147. Gurpinar E, Vousden KH (2015) Hitting cancers' weak spots: vulnerabilities imposed by p53 mutation. *Trends Cell Biol* 25: 486–495

148. Liu Y, Zhang X, Han C, Wan G, Huang X, Ivan C, Jiang D, Rodriguez-Aguayo C, Lopez-Berestein G, Rao PH *et al* (2015) TP53 loss creates therapeutic vulnerability in colorectal cancer. *Nature* 520: 697–701

149. Uchida C (2012) The retinoblastoma protein: functions beyond the G1-S regulator. *Curr Drug Targets* 13: 1622–1632

150. Dimova DK, Dyson NJ (2005) The E2F transcriptional network: old acquaintances with new faces. *Oncogene* 24: 2810–2826

151. Qin XQ, Livingston DM, Kaelin WG, Adams PD (1994) Deregulated transcription factor E2F-1 expression leads to S-phase entry and p53-mediated apoptosis. *Proc Natl Acad Sci USA* 91: 10918–10922

152. Stanelle J, Pützer BM (2006) E2F1-induced apoptosis: turning killers into therapeutics. *Trends Mol Med* 12: 177–185

153. Bertoli C, Skotheim JM, de Bruin RAM (2013) Control of cell cycle transcription during G1 and S phases. *Nat Rev Mol Cell Biol* 14: 518–528

154. Xu M, Sheppard KA, Peng CY, Yee AS, Piwnica-Worms H (1994) Cyclin A/CDK2 binds directly to E2F-1 and inhibits the DNA-binding activity of E2F-1/DP-1 by phosphorylation. *Mol Cell Biol* 14: 8420–8431

155. Chen YN, Sharma SK, Ramsey TM, Jiang L, Martin MS, Baker K, Adams PD, Bair KW, Kaelin WG (1999) Selective killing of transformed cells by cyclin/cyclin-dependent kinase 2 antagonists. *Proc Natl Acad Sci USA* 96: 4325–4329

156. Carrano AC, Eytan E, Hershko A, Pagano M (1999) SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nat Cell Biol* 1: 193–199

157. Binné UK, Classon MK, Dick FA, Wei W, Rape M, Kaelin WG, Näär AM, Dyson NJ (2007) Retinoblastoma protein and anaphase-promoting complex physically interact and functionally cooperate during cell-cycle exit. *Nat Cell Biol* 9: 225–232

158. Ji P, Jiang H, Rekhtman K, Bloom J, Ichetovkin M, Pagano M, Zhu L (2004) An Rb-Skp2-p27 pathway mediates acute cell cycle inhibition by Rb and is retained in a partial-penetrance Rb mutant. *Mol Cell* 16: 47–58

159. Wang H, Bauzon F, Ji P, Xu X, Sun D, Locker J, Sellers RS, Nakayama K, Nakayama KI, Cobrinik D *et al* (2010) Skp2 is required for survival of aberrantly proliferating Rb1-deficient cells and for tumorigenesis in Rb1+/− mice. *Nat Genet* 42: 83–88

160. Blain SW, Montalvo E, Massagué J (1997) Differential interaction of the cyclin-dependent kinase (Cdk) inhibitor p27Kip1 with cyclin A-Cdk2 and cyclin D2-Cdk4. *J Biol Chem* 272: 25863–25872

161. Lu Z, Bauzon F, Fu H, Cui J, Zhao H, Nakayama K, Nakayama KI, Zhu L (2014) Skp2 suppresses apoptosis in Rb1-deficient tumours by limiting E2F1 activity. *Nat Commun* 5: 3463

162. Potter CJ, Huang H, Xu T (2001) *Drosophila* Tsc1 functions with Tsc2 to antagonize insulin signaling in regulating cell growth, cell proliferation, and organ size. *Cell* 105: 357–368

163. Tapon N, Ito N, Dickson BJ, Treisman JE, Hariharan IK (2001) The *Drosophila* tuberous sclerosis complex gene homologs restrict cell growth and cell proliferation. *Cell* 105: 345–355

164. Kubota H (2009) Quality control against misfolded proteins in the cytosol: a network for cell survival. *J Biochem* 146: 609–616

165. Zhou X, Ikenoue T, Chen X, Li L, Inoki K, Guan K-L (2009) Rheb controls misfolded protein metabolism by inhibiting aggresome formation and autophagy. *Proc Natl Acad Sci USA* 106: 8923–8928

166. Li B, Gordon GM, Du CH, Xu J, Du W (2010) Specific killing of Rb mutant cancer cells by inactivating TSC2. *Cancer Cell* 17: 469–480

167. Gordon GM, Zhang T, Zhao J, Du W (2013) Deregulated G1-S control and energy stress contribute to the synthetic-lethal interactions between inactivation of RB and TSC1 or TSC2. *J Cell Sci* 126: 2004–2013

168. Nicolay BN, Gameiro PA, Tschöp K, Korenjak M, Heilmann AM, Asara JM, Stephanopoulos G, Iliopoulos O, Dyson NJ (2013) Loss of RBF1 changes glutamine catabolism. *Genes Dev* 27: 182–196

169. Cui M, Cohen ML, Teng C, Han M (2013) The tumor suppressor Rb critically regulates starvation-induced stress response in *C. elegans*. *Curr Biol* 23: 975–980

170. Reynolds MR, Lane AN, Robertson B, Kemp S, Liu Y, Hill BG, Dean DC, Clem BF (2014) Control of glutamine metabolism by the tumor suppressor Rb. *Oncogene* 33: 556–566

171. Crino PB, Nathanson KL, Henske EP (2006) The tuberous sclerosis complex. *N Engl J Med* 355: 1345–1356

172. Zhang T, Liao Y, Hsu F-N, Zhang R, Searle JS, Pei X, Li X, Ryoo HD, Ji J-Y, Du W (2014) Hyperactivated Wnt signaling induces synthetic lethal interaction with Rb inactivation by elevating TORC1 activities. *PLoS Genet* 10: e1004357

173. Napoli C, Sessa M, Infante T, Casamassimi A (2012) Unraveling framework of the ancestral Mediator complex in human diseases. *Biochimie* 94: 579–587

174. Dehainault C, Garancher A, Castéra L, Cassoux N, Aerts I, Doz F, Desjardins L, Lumbroso L, Montes de Oca R, Almouzni G *et al* (2014) The survival gene MED4 explains low penetrance retinoblastoma in patients with large RB1 deletion. *Hum Mol Genet* 23: 5243–5250

175. Cermelli S, Jang IS, Bernard B, Grandori C (2014) Synthetic lethal screens as a means to understand and treat MYC-driven cancers. *Cold Spring Harb Perspect Med* 4: 3

176. Sodir NM, Swigart LB, Karnezis AN, Hanahan D, Evan GI, Soucek L (2011) Endogenous Myc maintains the tumor microenvironment. *Genes Dev* 25: 907–916

177. Hueber AO, Zörnig M, Lyon D, Suda T, Nagata S, Evan GI (1997) Requirement for the CD95 receptor-ligand pathway in c-Myc-induced apoptosis. *Science* 278: 1305–1309

178. Klefstrom J, Verschuren EW, Evan G (2002) c-Myc augments the apoptotic activity of cytosolic death receptor signaling proteins by engaging the mitochondrial apoptotic pathway. *J Biol Chem* 277: 43224–43232

179. Wang Y, Engels IH, Knee DA, Nasoff M, Deveraux QL, Quon KC (2004) Synthetic lethal targeting of MYC by activation of the DR5 death receptor pathway. *Cancer Cell* 5: 501–512

180. Rottmann S, Wang Y, Nasoff M, Deveraux QL, Quon KC (2005) A TRAIL receptor-dependent synthetic lethal relationship between MYC activation and GSK3beta/FBW7 loss of function. *Proc Natl Acad Sci USA* 102: 15195–15200

181. Sato M, Rodriguez-Barrueco R, Yu J, Do C, Silva JM, Gautier J (2015) MYC is a critical target of FBXW7. *Oncotarget* 6: 3292–3305

182. Gorrini C, Squatrito M, Luise C, Syed N, Perna D, Wark L, Martinato F, Sardella D, Verrecchia A, Bennett S *et al* (2007) Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response. *Nature* 448: 1063–1067

183. Shreeram S, Hee WK, Demidov ON, Kek C, Yamaguchi H, Fornace AJ, Anderson CW, Appella E, Bulavin DV (2006) Regulation of ATM/p53-dependent suppression of myc-induced lymphomas by Wip1 phosphatase. *J Exp Med* 203: 2793–2799

184. Zhou Z, Patel M, Ng N, Hsieh MH, Orth AP, Walker JR, Batalov S, Harris JL, Liu J (2014) Identification of synthetic lethality of PRKDC in MYC-dependent human cancers by pooled shRNA screening. *BMC Cancer* 14: 944

185. Ferrao PT, Bukczynska EP, Johnstone RW, McArthur GA (2012) Efficacy of CHK inhibitors as single agents in MYC-driven lymphoma cells. *Oncogene* 31: 1661–1672

186. Höglund A, Nilsson LM, Muralidharan SV, Hasvold LA, Merta P, Rudelius M, Nikolova V, Keller U, Nilsson JA (2011) Therapeutic implications for

the induced levels of Chk1 in Myc-expressing cancer cells. *Clin Cancer Res* 17: 7067–7079

187. den Hollander J, Rimpi S, Doherty JR, Rudelius M, Buck A, Hoellein A, Kremer M, Graf N, Scheerer M, Hall MA *et al* (2010) Aurora kinases A and B are up-regulated by Myc and are essential for maintenance of the malignant state. *Blood* 116: 1498–1505

188. Yang D, Liu H, Goga A, Kim S, Yuneva M, Bishop JM (2010) Therapeutic potential of a synthetic lethal interaction between the MYC proto-oncogene and inhibition of aurora-B kinase. *Proc Natl Acad Sci USA* 107: 13836–13841

189. Gustafson WC, Meyerowitz JG, Nekritz EA, Chen J, Benes C, Charron E, Simonds EF, Seeger R, Matthay KK, Hertz NT *et al* (2014) Drugging MYCN through an allosteric transition in Aurora kinase A. *Cancer Cell* 26: 414–427

190. Goga A, Yang D, Tward AD, Morgan DO, Bishop JM (2007) Inhibition of CDK1 as a potential therapy for tumors over-expressing MYC. *Nat Med* 13: 820–827

191. Kang J, Sergio CM, Sutherland RL, Musgrove EA (2014) Targeting cyclin-dependent kinase 1 (CDK1) but not CDK4/6 or CDK2 is selectively lethal to MYC-dependent human breast cancer cells. *BMC Cancer* 14: 32

192. Kessler JD, Kahle KT, Sun T, Meerbrey KL, Schlabach MR, Schmitt EM, Skinner SO, Xu Q, Li MZ, Hartman ZC *et al* (2012) A SUMOylation-dependent transcriptional subprogram is required for Myc-driven tumorigenesis. *Science* 335: 348–353

193. Hsu TY-T, Simon LM, Neill NJ, Marcotte R, Sayad A, Bland CS, Echeverria GV, Sun T, Kurley SJ, Tyagi S *et al* (2015) The spliceosome is a therapeutic vulnerability in MYC-driven cancer. *Nature* 525: 384–388

194. Sonenberg N, Hinnebusch AG (2009) Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* 136: 731–745

195. Pourdehnad M, Truitt ML, Siddiqi IN, Ducker GS, Shokat KM, Ruggero D (2013) Myc and mTOR converge on a common node in protein synthesis control that confers synthetic lethality in Myc-driven cancers. *Proc Natl Acad Sci USA* 110: 11988–11993

196. Lin C-J, Nasr Z, Premsrirut PK, Porco JA, Hippo Y, Lowe SW, Pelletier J (2012) Targeting synthetic lethal interactions between Myc and the eIF4F complex impedes tumorigenesis. *Cell Rep* 1: 325–333

197. Fernández-Medarde A, Santos E (2011) Ras in cancer and developmental diseases. *Genes & Cancer* 2: 344–358

198. Rajalingam K, Schreck R, Rapp UR, Albert S (2007) Ras oncogenes and their downstream targets. *Biochim Biophys Acta* 1773: 1177–1195

199. Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM (2013) K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature* 503: 548–551

200. Steckel M, Molina-Arcas M, Weigelt B, Marani M, Warne PH, Kuznetsov H, Kelly G, Saunders B, Howell M, Downward J *et al* (2012) Determination of synthetic lethal interactions in KRAS oncogene-dependent cancer cells reveals novel therapeutic targeting strategies. *Cell Res* 22: 1227–1245

201. Gilad O, Nabet BY, Ragland RL, Schoppy DW, Smith KD, Durham AC, Brown EJ (2010) Combining ATR suppression with oncogenic Ras synergistically increases genomic instability, causing synthetic lethality or tumorigenesis in a dosage-dependent manner. *Cancer Res* 70: 9693–9702

202. Dietlein F, Kalb B, Jokic M, Noll EM, Strong A, Tharun L, Ozretić L, Künstlinger H, Kambartel K, Randerath WJ *et al* (2015) A Synergistic Interaction between Chk1- and MK2 Inhibitors in KRAS-Mutant Cancer. *Cell* 162: 146–159

203. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, Kris MG, Miller VA, Ladanyi M, Riely GJ (2013) Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res* 19: 2240–2247

204. Unni AM, Lockwood WW, Zejnullahu K, Lee-Lin S-Q, Varmus H (2015) Evidence that synthetic lethality underlies the mutual exclusivity of oncogenic KRAS and EGFR mutations in lung adenocarcinoma. *eLife* 4: e06907

205. Petti C, Molla A, Vegetti C, Ferrone S, Anichini A, Sensi M (2006) Coexpression of NRASQ61R and BRAFV600E in human melanoma cells activates senescence and increases susceptibility to cell-mediated cytotoxicity. *Cancer Res* 66: 6503–6511

206. Vicent S, Chen R, Sayles LC, Lin C, Walker RG, Gillespie AK, Subramanian A, Hinkle G, Yang X, Saif S *et al* (2010) Wilms tumor 1 (WT1) regulates KRAS-driven oncogenesis and senescence in mouse and human models. *J Clin Invest* 120: 3940–3952

207. Wang Y, Ngo VN, Marani M, Yang Y, Wright G, Staudt LM, Downward J (2010) Critical role for transcriptional repressor Snail2 in transformation by oncogenic RAS in colorectal carcinoma cells. *Oncogene* 29: 4658–4670

208. McLornan DP, List A, Mufti GJ (2014) Applying synthetic lethality for the selective targeting of cancer. *N Engl J Med* 371: 1725–1735

209. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E, Scholl C *et al* (2009) Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature* 462: 108–112

210. Puyol M, Martín A, Dubus P, Mulero F, Pizcueta P, Khan G, Guerra C, Santamaría D, Barbacid M (2010) A synthetic lethal interaction between K-Ras oncogenes and Cdk4 unveils a therapeutic strategy for non-small cell lung carcinoma. *Cancer Cell* 18: 63–73

211. Mao C-Q, Xiong M-H, Liu Y, Shen S, Du X-J, Yang X-Z, Dou S, Zhang P-Z, Wang J (2014) Synthetic lethal therapy for KRAS mutant non-small-cell lung carcinoma with nanoparticle-mediated CDK4 siRNA delivery. *Mol Ther* 22: 964–973