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Review

Synthetic Lethality-based Targets for Discovery of New Cancer Therapeutics

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Abstract. *Synthetic lethality is based on the incompatibility of cell survival with the loss of function of two or more genes, not with loss of function of a single gene. If targets of synthetic lethality are deregulated or mutated in cancer cells, the strategy of synthetic lethality can result in significant increase of therapeutic efficacy and a favourable therapeutic window. In this review, we discuss synthetic lethality based on deficient DNA repair mechanisms, activating mutations of RAS, loss of function mutations of the tumor suppressor genes p53, Rb and von Hippel-Lindau, and disruption of interactive protein kinase networks in the context of development of new anticancer agents.*

The most significant achievements in the development of anticancer drugs are dependent on the understanding of the biology of the disease (1). Especially promising are approaches which focus on targets of tumor subtypes, in contrast to their corresponding normal cells, with impact on potential significant therapeutic benefit and a favourable therapeutic window. A breakthrough in this context was the discovery of imatinib (Gleevec), a kinase inhibitor of the constitutively active fusion protein consisting of BCR and ABL (BCR–ABL) for the treatment of chronic myeloid leukemia (CML) (2). This concept was extended to the treatment of non-small cell lung carcinoma (NSCLC) expressing epidermal growth factor receptor (EGFR) with EGFR inhibitors, gastrointestinal stromal tumor (GIST) with tyrosine kinase KIT (CD117) inhibitors, melanomas with the activating V600E B-rat fibrosarcoma (*BRAF*) mutation with a kinase inhibitor and NSCLC patients with the echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4–ALK) fusion protein with an ALK inhibitor (3, 4). A promising concept of anticancer drug development is based on synthetic lethality (SL), which arises when a combination of inactivating mutations in two or more genes results in cell death, whereas a mutation in one of the genes does not (5). The concept was pioneered by screening chemical libraries that specifically kill yeast deletion mutants with defects in cell-cycle checkpoints or DNA repair (6, 7). Many SL interactions have been identified by making use of interference screens in mammalian and human cells (8-11). Figure 1 describes the scenarios for discussed in this review. The context can be a mutated oncogene (Figure 1A), a mutated tumor suppressor gene (Figure 1B), an overexpressed oncogene (Figure 1C), two genes as part of a protein kinase network (Figure 1D), or genes belonging to the class of DNA mismatch repair genes and a second gene involved in either DNA repair or DNA synthesis (Figure 1E). Scenarios A and C rely on loss of function in only one of the two involved genes.

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RAS-related SL

Ki-rat sarcoma gene *(KRAS)* is mutated in 30% of human tumors with a prevalence of >90% in pancreatic adenocarcinoma. Therefore, the identification of vulnerabilities of human tumors expressing oncogenic Ki-RAS is an important issue. Large-scale RNA interference (RNAi) screens can be used for identification of such vulnerabilities. Cells that are dependent on mutant Ki-RAS exhibit sensitivity to suppression of the serine-threonine kinase 33 (STK33) irrespective of tissue origin, whereas Ki-RAS independent cells do not require STK33 (12). STK33 selectively promotes viability in Ki-RAS dependent cells in a kinase-dependent manner by suppression of mitochondrial apoptosis by p70 ribosomal protein S6 kinase 1 S6K (*RPS6KB1*)-induced

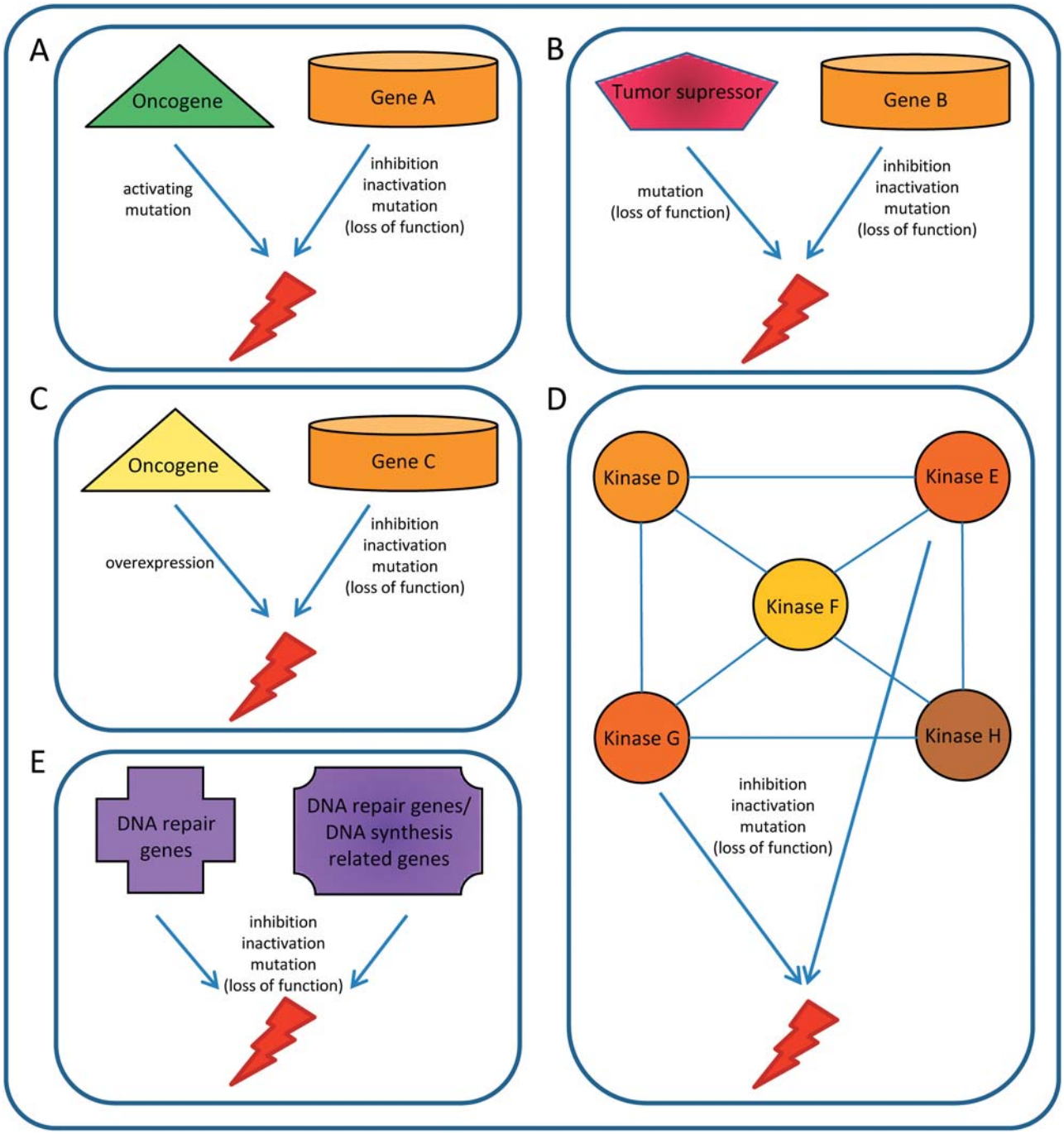

Figure 1. *Scenarios for synthetic lethality. Possible molecular interactions leading to synthetic lethality are summarized: A: Activated oncogene and inactivation of another gene, B: inactivated tumor suppressor gene and inactivation of another gene, C: overexpression of an oncogene and inactivation of another gene, D: inactivation of two kinases as part of a signaling network, E: inactivation of a pair of DNA repair genes and DNA synthesis-related/DNA repair genes. Red arrows indicate synthetic lethality.*

inactivation of the proapoptotic *BCL2*- associated death promoter (BAD) selectively in Ki-RAS-dependent cells. Tumor cells with mutated *NRAS* or *HRAS* do not exhibit this phenomenon. STK33 is not a component of the RAS signaling pathway and does not serve as an oncogene on its own in transformation assays. The lethal interaction between Ki-RAS and STK33 is restricted to cells which are functionally dependent on Ki-RAS, therefore it is important to identify

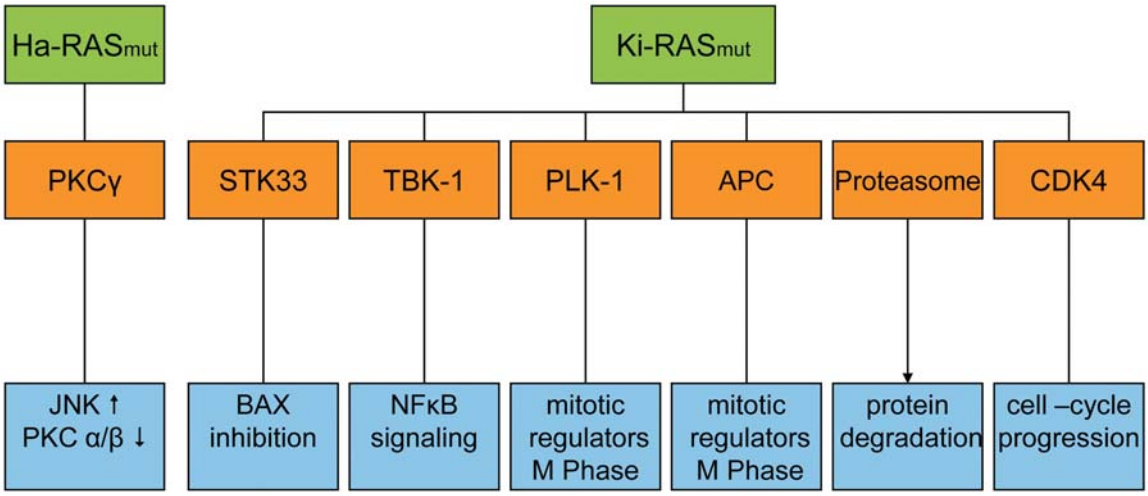

Figure 2. *RAS-mediated synthetic lethality. The second row displays target molecules for Ha-RAS- and Ki-RAS-based synthetic lethality and the third row shows the physiological consequences of the inhibition of the effector molecules or the pathway involved. APC: Anaphase-promoting complex; BAX: BCL-2-mediated X protein; CDK4: cyclin-dependent kinase 4; JNK: jun N-terminal kinase; NFκB: nuclear factor κB; PKC α, β, γ: protein kinase C α, β or γ; STK 33: serine/threonine kinase 33; TBK-1: TANK-binding kinase 1. Green boxes and brown boxes highlight the targets involved in synthetic lethal partnerships and blue boxes indicate the mode of action (MOA) responsible for synthetic lethality.*

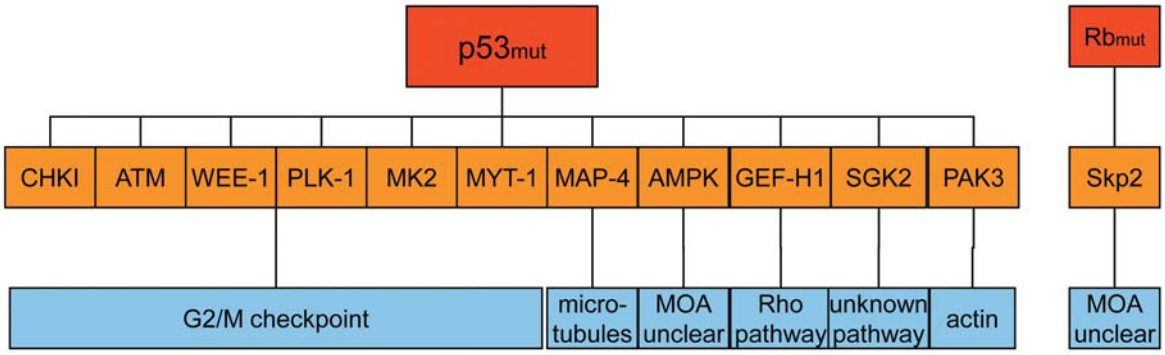

Figure 3. *p53 mutant- and Rb mutant-mediated synthetic lethality. The p53 and Rb mutants are all loss-of-function mutations. Target molecules for synthetic lethality are shown in the second row and pathways or targets involved in synthetic lethality are shown in the third row. AMPK: AMP-activated protein kinase; ATM: ataxia telangiectasia mutated; CHK1: checkpoint kinase 1; GEF-H1: guanine nucleotide exchange factor-H1; MAP4: microtubule-associated protein 4; MK2: MAP-kinase activated protein kinase 2; MYT-1: dual-specificity kinase; PAK3: serine/threonine kinase; PLK1: polo-kinase 1; SGK2: serine/threonine kinase; Skp2: S-phase kinase associated protein 2; Wee1: nuclear serine/threonine kinase 1. Red and brown boxes highlight the partners involved in a synthetic lethal partnership and blue boxes refer to the MOA for synthetic lethality.*

characteristics which mediate the dependency of primary tumors on Ki-RAS. For the development of anticancer agents based on the lethal interaction between Ki-RAS and STK33, the elucidation of the physiological role of STK33 is important to define possible toxicity-related liabilities.

A genome-wide RNAi screen was performed to identify genes whose inhibition results in reduced viability of Ki-RAS mutant cells (13). The identified targets included cyclin A2 (CCNA2), kinesin-like protein 2C (KIF2C), polo-like kinase 1 (PLK1) and anaphase-promoting complex/cyclosome (APC/C), indicating that Ki-RAS cells are vulnerable to mitotic perturbations. As predicted from the RNAi analysis, Ki-RAS mutant cells are preferentially killed by mitotic spindle function targeting drugs such as paclitaxel, a PLK1 inhibitor and the proteasome inhibitor bortezomib. Microarray analysis of lung adenocarcinomas showed an inverse correlation between the mitotic machinery genes with patient survival in a Ki-RAS status-dependent manner. All of these proteins are required by normal cells as well; therefore the difference between Ki-RAS mutant cells and wild-type Ki-RAS is quantitative, not

qualitative. Making use of RNAi screening in a cell panel composed of wild-type and mutant Ki-RAS cells, TANK-binding kinase (TBK1) was also identified as a synthetic lethal gene (14). TBK1 is a non-canonical inhibitor of κB protein (IκB) that is known to regulate nuclear factor κB (NFκB) signaling. These results are based on screening immortalized lung epithelial cells (mutant Ki-RAS *versus* control), 19 cancer cell lines in the primary screen and 11 NSCLC cell lines in the secondary screen, and were confirmed in xenografts *in vivo*: 2 Ki-RAS wild-type and 2 mutant Ki-RAS.

In a mouse model of NSCLC, an unexpected lethal interaction between mutant Ki-RAS and cyclin-dependent kinase (CDK) ablation was detected (15). Genetic ablation of CDK4 (but not of CDK2 or CDK6) prevented development of Ki-RAS-induced NSCLC development *in vivo*. Mutant Ki-RAS was also induced in other tissues such as pancreas and colon, but these tissues do not undergo hyperplasia or develop tumors and senescence was not induced in the absence of CDK4. The results indicate that induction of senescence might be specific for proliferating tissues and therefore might be selective for tumor cells. It remains to be seen whether inhibition of CDK4 in NSCLC with mutant Ki-RAS will translate into clinical benefit.

A synthetic lethal interaction between loss of protein kinase Cδ (PKCδ) and mutated *HRAS* has been described (16) with NIH3T3/Ha-RAS and DU145 prostate cancer cells as experimental systems. During the apoptotic process, PKCα and β are up-regulated and then associated with receptor of activated protein kinase C1 (RACK1), an adaptor for activated PKCδ and jun *N*-terminal kinase (JNK). Immunoblotting revealed that JNK is phosphorylated, accompanied by caspase 8 cleavage. The inhibition of JNK abrogates this process triggered by PKCδ knockdown. The data suggest that PKCα, β and PKCδ function oppositely to maintain a balance supporting Ha-RAS cells to survive. Selective killing of Ki-RAS mutated colon cancer cells was reported (17). Ki-RAS mutated colon cancer cells engineered to produce blue fluorescent protein were co-cultured with a subclone in which the mutant Ki-RAS allele was eliminated by homologous recombination and engineered to produce yellow fluorescent protein. Several chemical entities, among them a novel cytidine nucleotide, were identified as selective killers of Ki-RAS mutant colon cancer cells. A screen in isogenic HCT116 colon carcinoma cells with a library targeting 2500 genes revealed that the transcription factor SNAIL-2 is only required in RAS mutant cells (18, 19). The SL interactions between mutated Ha-RAS, Ki-RAS and other partners are summarized in Figure 2.

p53 and Rb-related SL

The corresponding lethal interactions are summarized in Figure 3. Protein p53 (p53) is mutated in 50% of human tumors, therefore SL in the context of p53 loss of function is an important issue for future anticancer drug development. However, one has to keep in mind that not all mutants result in a complete loss of function phenotype (20-22). Original attempts included screening of the 60 National Cancer Institute (NCI) cancer cell lines for compounds which selectively kill cells devoid of p53 (23). Few classes of compounds were identified, including paclitaxel. Subsequently, microtubule-associated protein-4 (MAP-4), a p53 transcriptional repressed target, was identified as a possible mediator of susceptibility to paclitaxel killing (24). PLK1, a regulator of the G_2/M checkpoint was identified as consistently upregulated in four paired colon cancer cell lines isogenic for p53 deletion or mutation (25). p53-deficient cell lines were more sensitive to PLK1 inhibitors and a PLK1 inhibitor as a single agent caused regression of *p53* null tumors. Another approach focuses on compound screening in a p53 temperature-sensitive model killing cells at 39˚C (mutant p53 status), but not at 32˚C (wild-type p53 status) (26). Vulnerability of p53 deficient tumor cell lines *versus* metformin, a diabetic drug, was described (27). The mechanism of killing is based on inhibition of oxidative phosphorylation and activation of AMP-activated protein kinase (AMPK) which creates an environment more vulnerable to p53 mutant cells. In p53 mutant cells, the G_1 checkpoint is abrogated due to lack of p53-mediated *p21* induction in response to DNA damage. Further abrogation of the G_2 checkpoint control will selectively kill p53-deficient cancer cells through induction of mitotic catastrophe. UCN01, a G_2 checkpoint abrogator, sensitizes p53 mutant cancer cell lines to irradiation abrogating G_2 checkpoint control (28). Nuclear serine/threonine family kinase *WEE-1* is a DNA damage-induced kinase involved in the G_2/M checkpoint preventing cell-cycle progression through inactivation of the CDK1 by phosphorylation (29). Inhibition of *WEE-1* exerts a cytotoxic effect on p53 deficient tumor cells (30). Similar observations were made through knock-down experiments for other G_2/M checkpoint regulators such as checkpoint kinase 1 (CHK1), dual specificity kinase (*MYT1*) and serine-threonine kinase ataxia telangiectasia mutated *(ATM)* (31, 32). Mutant p53 was shown to induce the *GEF-H1* oncogene, a guanine nucleotide exchange factor-H1 for RAS homology gene family member A (*RHOA*) resulting in accelerated proliferation of tumor cells and therefore is a possible target for an SL-related approach (33). Synthetic lethal interaction between p53 and the serine/threonine protein kinases SGK2 and PAK3 was observed making use of a panel of 100 hairpin RNAs targeting essential kinases in human papilloma virus (HPV)-transformed human cervical carcinoma cells (34). SL was mediated as a consequence of p53 inactivation by HPV E6 protein (HPV E6). The experiments suggest that the mechanisms of SL are different for the two kinases: SGK2 depletion caused autophagy, whereas PAK3 depletion caused caspase 3 activation. These findings suggest that SGK2 and

PAK3 are not components of the same pathway but represent independent types of sensitivities. The results do not indicate whether kinase activity of SGK2 and PAK3 is important for SL after p53 loss. PAK3 has been implicated in actin filament regulation in proliferating cells (35); for SGK2 no function-related data are available. Silencing of MAP kinase-activated protein kinase 2 (MK2) was shown to exhibit SL in the context of p53 deficiency in the presence of DNA damage (36). MK2 depletion decreased the phosphorylation level of dual specificity phosphatase Cdc 25A/B (mammalian homologues of the *S. pombe* 25A and 25B genes) providing initial evidence that MK2 plays an important role in cell-cycle checkpoints. Another SL relationship was uncovered for retinoblastoma protein (Rb) and S-phase kinase-associated protein 2 (Skp2) (37). Knock-out of the *pRb* target Skp2 in $pRb^{+/-}$ mice induced apoptosis and completely inhibited tumorigenesis in the pituitary intermediate lobe. Skp2 is a substrate-recruiting component of the Skp, Cullin, F-Box containing complex SCF (Skp2) E3 ubiquitin ligase. One of its substrates is Thr187 phosphorylated p27 (Kip1). A *p27*T187A mutation (knock-in) phenocopied *Skp2* knock-out in inducing apoptosis following Rb loss. Thus *Skp2* knock-out or *p27*T187A knock-in are synthetically lethal together with *pRb* inactivation.

Protein Kinase Network and SL

An example of an approved drug exploiting SL is the rapamycin derivative CCI-779, which exhibits enhanced activity against tumors with mutations in phosphatase and tensin homologue (*PTEN*) compared to tumors with wild-type *PTEN*. Target of CCI-779 is the protein kinase mTOR which acts downstream of the phosphoinositide 3-kinase-AKT (PI3K-AKT) pathway which is up-regulated in PTEN-deficient tumors (38). EGFR/Notch signaling seem to be essential parts of a protein kinase network in basal-like breast cancer (BLBC). 15% of the breast carcinomas are of the BLBC subtype and are characterized by absence of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), EGFR and Notch-signaling are frequently activated in this type of cancer. Inhibition of EGFR or Notch signaling alone is insufficient to suppress BLBC tumor cell survival and proliferation; simultaneous suppression has uncovered a synthetic lethal relationship between the two oncogenic pathways (39). The relationship is due to a decrease in AKT activation after simultaneous pathway inhibition. Expression of an activated form of Notch-1 was shown to restore AKT activity after dual pathway blockade. Combined inhibition of both pathways also resulted in dramatic improvement of *in vivo* activity. A synthetic lethal approach for treating glioblastoma was described (40). Amplification of *EGFR* or truncation mutant of EGFR plays an important role in the pathogenesis of glioblastoma (41). Suppression of both EGFRvIII and AKT-2 significantly inhibited tumor growth by inducing apoptosis and prolonged the duration of median survival in a glioblastoma xenograft model from 14 to 31.5 d. However, it was not shown whether knock-down of *EGFR*vIII suppresses AKT activity. Also there is no explanation for the observation that SL is specific for the knock-down of *AKT-2* and not for *AKT-1* and *AKT-3* keeping in mind that these molecules have similar functions. The issue of context-specificity of the observed phenomenon has also not been investigated. The latter issue can be resolved by evaluation of cell lines with different genetic background and corresponding knock-down experiments. A striking SL relationship has been revealed in the context of studying insulin-like growth factor (IGFR-1) inhibitor-resistant childhood sarcomas (42). The experiments revealed that BMS-536924 resistant cell lines are sensitive to loss of distal rather than proximal IGFR-1 signaling components, such as ribosomal protein S6 (RPS6), and that BMS-536924 fails to block RPS6 activation in resistant sarcoma cell lines and that knock-out of macrophage-stimulating 1 receptor tyrosine kinase (MST1R, also known as RON), restores BMS-536924 efficacy even in highly resistant cell lines. It was found that loss of MST1R by RNAi blocks downstream RPS6 activation. This is just another example of the exploitation of protein kinase networks for the identification of a synthetic lethal relationship. The protein kinase network based SL interactions are summarized in Figure 4.

MYC-related SL

Myelocytomatosis viral oncogene homolog *MYC* is a transcription factor overexpressed in many types of neoplasia, but its tractability with pharmacologic agents is a problematic issue. An alternative is to exploit synthetic lethal interactions between overexpressed MYC and other targets in tumor cells, leaving non-transformed cells unaffected (43-45). Anti-malaria agent chloroquine showed MYC-selective killing of tumor cells including autophagy resulting in induction of p53 dependent apoptosis (46). Cell death was not impaired by a genetic deficiency of autophagy-related protein (atg7). Chloroquine appeared to be active as a preventive agent in a mouse model of B-cell lymphoma, but did not mediate tumor growth inhibition as a single agent. Overexpression of MYC sensitizes fibroblasts to agonists of the death receptor DR5 (47). It was shown that MYC mediates increased DR5 expression and signaling as a result of enhanced procaspase 8 autocatalytic activity. Screening of a library of small RNAs revealed a synthetic lethal interaction between MYC activation and glycogen synthase kinase 3β (GSK 3β) whose inactivation potentiates TNF-related apoptosis-inducing ligand (TRAIL) specifically in MYC overexpressing cells (48). Small RNA-mediated silencing of GSK-3β prevents

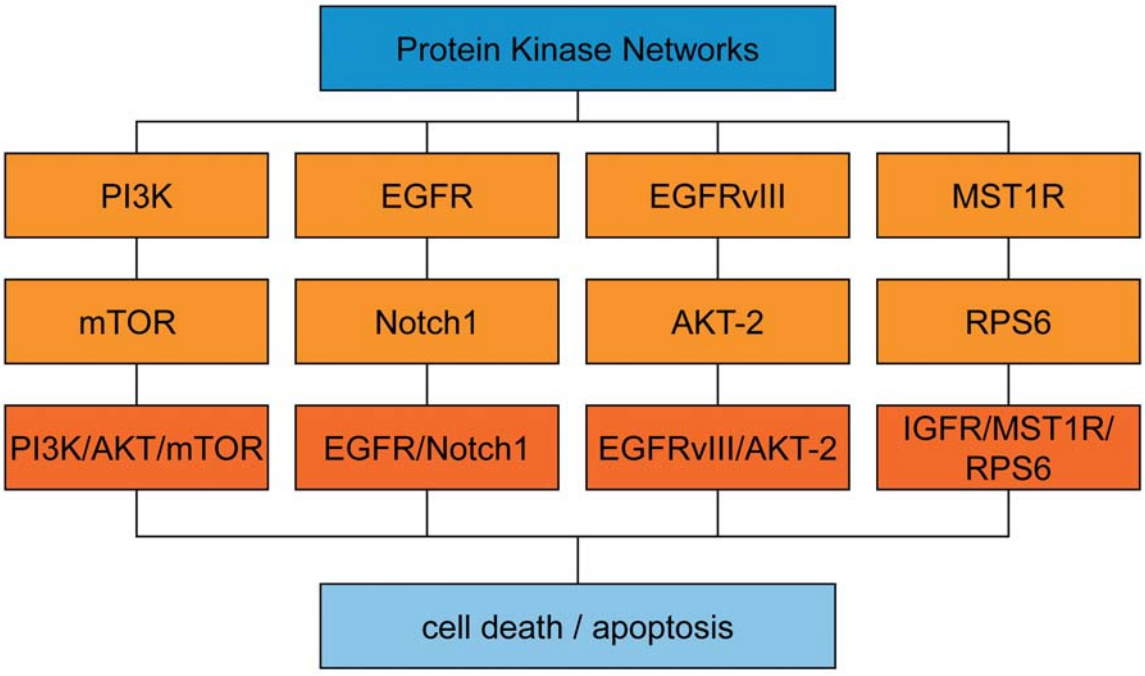

Figure 4. *Synthetic lethality with involvement of protein kinase networks. First and second rows highlight targets and pathways involved in the synthetic lethal interaction, the third row shows the final synthetic lethal interactions. EGFRvIII: Epidermal growth factor receptor variant III; IGFR: insulin-like growth factor receptor; MST1R: macrophage-stimulating 1 receptor tyrosine kinase; mTOR: mammalian target of rapamycin; PI3K: phosphoinositide 3 kinase; RPS6: ribosomal protein 6. Dark blue, and light and dark brown boxes highlight the context as well as the partners involved in synthetic lethal partnerships and the box in light blue indicates the MOA of the synthetic lethal partnerships.*

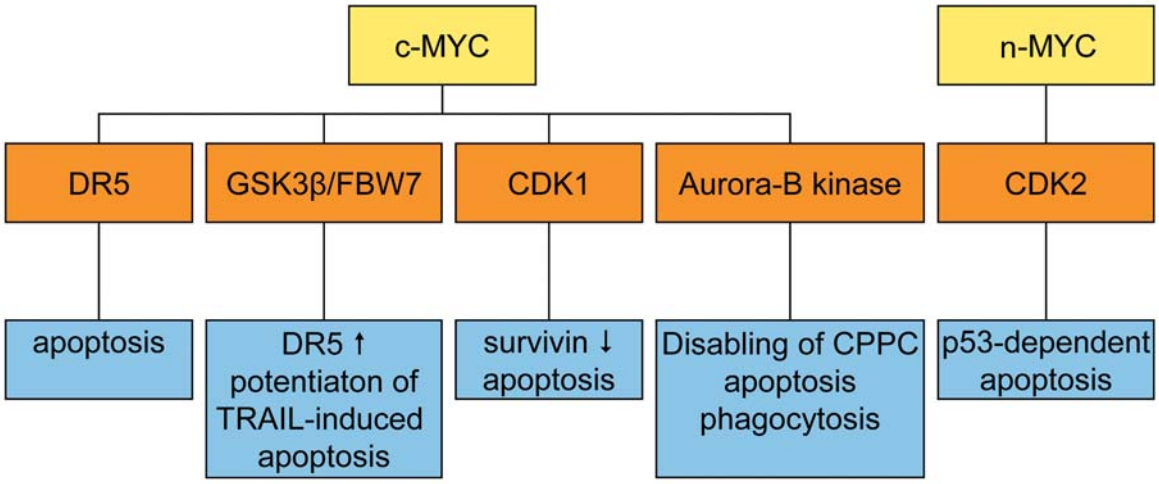

Figure 5. *MYC-related synthetic lethal interactions. The second row displays targets which are involved in synthetic lethality together with c-MYC or n-MYC and the third row highlights the underlying MOA. c-MYC and N-MYC are transcription factors. CDK1,2: cyclin-dependent kinases 1 and 2; CPPC: chromosomal passenger protein complex; FBW7: Fbox/WD repeat-containing protein 7; GSK3β: glycogen synthase kinase 3β. Yellow and brown boxes indicate the partners forming a synthetic lethal partnership and blue boxes show the MOA for synthetic lethality.*

phosphorylation of MYC on T58, inhibiting recognition of MYC by the E3 ubiquitin ligase component Fbox/WD repeat containing protein 7 (FBW7). This results in stabilization of MYC, up-regulation of TRAIL death receptor 5 (*DR5*) and potentiation of DR5 induced apoptosis *in vitro* and *in vivo*. These findings identify GSK-3β and FBW7 as cancer-related targets and demonstrate paradoxically that up-regulation of *c-MYC* can result in a preclinical therapeutic benefit. Mutations

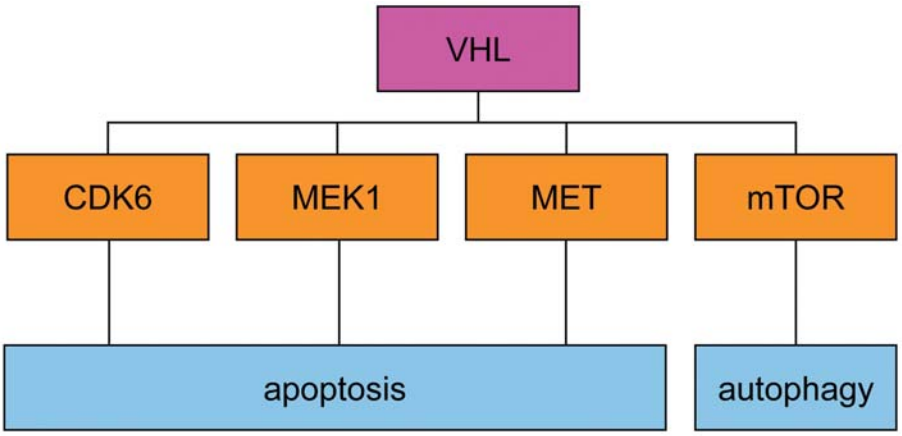

Figure 6. *VHL-mediated synthetic lethality. The rows show targets of VHL for synthetic lethality and the mode of killing of affected cancer cells. CDK6: Cyclin-dependent kinase 6; MEK1: mitogen-activated protein kinase 1; MET: hepatocyte growth factor receptor; mTOR: mammalian target of rapamycin. Pink and brown boxes indicate the partners forming a synthetic lethal partnership and the blue boxes refer to the MOA for synthetic lethality.*

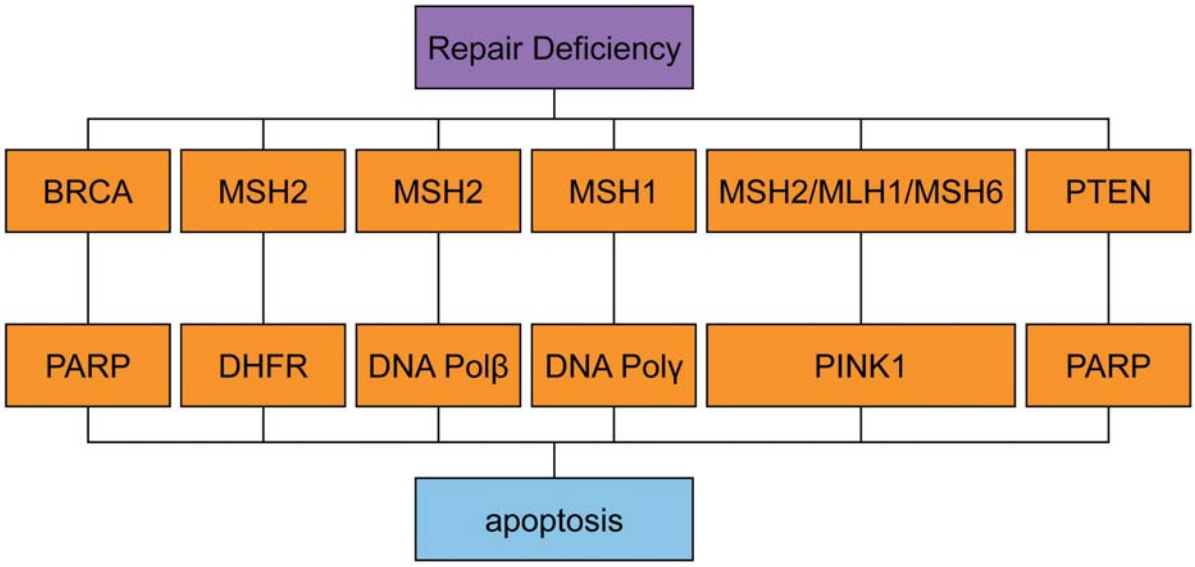

Figure 7. *Repair-deficiency based synthetic lethality. The rows show the partners for synthetic lethality; loss of function of the corresponding partner molecules results in synthetic lethality. BRCA: Breast cancer susceptibility protein; DHFR: dihydrofolate reductase; DNA Pol β and γ: DNA polymerase β and γ; MSH1, MSH2, MSH6, MLH1: mismatch-repair proteins; PARP: poly (ADP-ribose) polymerase; PINK1: PTEN-induced putative kinase 1; PTEN: phosphatase and tensin homolog. Purple and brown boxes indicate the partners forming synthetic lethal relationships and the blue box highlights the consequence of the synthetic lethal partnership.*

in the T58 GSK-3β phosphorylation site of *MYC* and inhibition of its degradation by the proteasome has been described in Burkitt's lymphoma (49, 50), whereas inactivating mutations of *FBW7* have been reported in several types of tumors including breast, ovarian, endometrial, pancreatic and colorectal carcinomas (51). These mutations correlate with *in vitro* responsiveness to TRAIL agonists and it will be of interest whether they can be used for patient stratification (48). Overexpression of MYC mediates sensitization to apoptosis when CDK1 is inhibited genetically or pharmacologically (52). It was shown that CDK1 inhibition leads to depletion of survivin thus resulting in apoptosis. CDK2 is often overexpressed in neuroblastomas with poor prognosis and a SL relationship was demonstrated with *n-MYC* amplification (53). Genetic and pharmacologic disruption of CDK2 results in p53-dependent apoptosis in *n-MYC* amplified neuroblastomas. A synthetic lethal interaction has been described between cells that overexpress c-MYC

and aurora B kinase (54). Exposure of cells to aurora B kinase inhibitor VX-680 selectively kills cells that overexpress c-MYC. Supporting RNAi experiments have shown that the effect is due to inhibition of aurora B kinase and consequent disabling of the chromosomal passenger protein complex (CPPC) and ensuing DNA replication in the absence of cell division. Bimodal killing due to apoptosis and autophagy is not dependent on p53. Efficacy of VX-680 was demonstrated in mouse models of B- and T-cell lymphomas induced by c-MYC as a transgene. Overexpression of c-MYC might be a biomarker for cells which are sensitive to VX-680. Independently it was reported that aurora kinases A and B are up-regulated by *MYC* and are essential for the maintenance of the malignant state (55). Corresponding SL interactions are summarized in Figure 5.

von Hippel-Lindau (VHL) and SL

Molecular and genetic studies have revealed the loss of the *VHL* gene as a frequent and crucial event for the pathogenesis of clear cell renal carcinoma. Due to the high mortality rate associated with this type of cancer, exploitation of this finding for drug development seems to be a straight-forward approach. VHL is a component of a protein complex that includes elongin B, elongin C and cullin 2 and possesses E3 ubiquitin ligase activity (56, 57). The *VHL* gene product is involved in ubiquitination and degradation of hypoxia-inducible factor (HIF) which is a transcription factor that plays a central role in regulation of gene expression by oxygen. Using publicly available data and analytical tools, chromomycin A3 (ChA3) was identified as an agent that showed genotype-selective toxicity against VHL-deficient cells (58). Co-culture of VHL-deficient and VHL-positive cells showed selective killing of the VHL-deficient cells. Mechanistically ChA3 is known to bind to the minor groove of DNA interfering with replication and transcription. ChA3 does not seem to mimic the action of VHL, because it had little effect on HIF-1α levels. A high-throughput screening approach identified STF-6224 as an agent with selective toxicity for VHL-deficient renal cancer cells (59, 60). It was demonstrated that the cytotoxicity of STF-62247 is due to deregulated autophagy. The reduction of protein levels of autophagy-related genes *Atg5, Atg7* and *Atg9* reduce the sensitivity of VHL-deficient cells to killing by STF-62247. This compound induced the formation of large intracytoplasmic vacuoles characteristic of cells undergoing autophagy, which is a lysosomal degradation pathway that regulates turnover of organelles and long-lived proteins. There is no evidence for apoptosis as a mediator of cell death since there was no increase in DNA condensation, increase in annexin V/propidium iodide or activation of caspase 3. Making use of yeast genetics it was shown that trafficking through the Golgi apparatus is an important pathway in STF-62247-induced autophagic toxicity. Several genes important in Golgi trafficking were identified as VHL targets. $VHL^{-/-}$ cells display increased sensitivity to mTOR (mammalian target of rapamycin) inhibition which can also promote autophagy (61). Making use of a synthetic lethal screen it was shown that CDK6 is required for the survival of VHL-negative cells (62). Sensitization to inhibition to mitogen activated protein kinase kinase 1 (MEK1) and hepatocyte growth factor receptor MET was observed. Corresponding SL interactions are summarized in Figure 6.

Repair-related Lethality

DNA can be damaged by chemicals, chemotherapy, UV-irradiation, ionizing radiation and reactive species derived from cellular and oxidative metabolism. Single-strand breaks can be repaired through base excision repair (BER), bulky adducts through nucleotide excision repair (NER) and mismatches through insertion or deletion by mismatch repair (MMR) (63, 64). Double-strand breaks are repaired by homologous recombination (HR), a process that restores the original DNA sequence, or by non-homologous end-joining (NHEJ) or single-strand annealing (SSA) (63, 64). The latter processes lack fidelity to germ-line sequence.

Poly (ADP-ribose) polymerase (PARP) and breast cancer susceptibility (BRCA) proteins are essential for the repair of DNA breaks and when defective, lead to the accumulation of mutations introduced by error-prone DNA repair. BRCA1 and BRCA2 regulate repair of damaged DNA through HR (65). At the site of double-stranded DNA damage, one strand of the DNA is degraded creating a stretch of single-stranded DNA which is bound by RAD51 (eukaryotic homolog of prokaryotic RecA protein) forming filamentous structures (66). The RAD51 foci promote recognition of homologous sequences on the sister chromatid and catalyse pairing between complimentary sequences. PARP is a nuclear protein that is activated when DNA is damaged (67, 68). Of the six PARP enzymes, PARP1 is essential for repair of single-strand breaks predominantly through the BER mechanism (69). Continuous inhibition of PARP leads to double-stranded breaks during DNA replication resulting in stalling of replication forks at the location of DNA damage. Repair of double-stranded DNA breaks depends on the HR pathway (69). The corresponding SL interactions are summarized in Figure 7. The MMR pathway is involved in removal of DNA base mismatches which arise during DNA replication or are introduced by DNA damage. Four gene products are essential for MMR: MSH2, MLH1, PMS2 and MSH6. Mutations in these predispose to hereditary nonpolyposis colon cancer (Lynch syndrome) (70). DNA damage is recognized by the components of the MMR pathway. The MutSα (MSH2/MSH6) heterodimer recognizes single-base mismatches and small insertion-deletion loops (IDL). The

MutSβ (MSH2/MSH3) heterodimer recognizes single-nucleotide IDL and longer IDLs. The mismatches are associated with MutL, which is recognized by MutS, are excised and repaired. Details of the mammalian MMR as a post-replicative process are described in (71).

BRCA and PARP Inhibition-mediated SL

BRCA deficient cells are 1000-fold more sensitive to single agent PARP inhibition than are wild-type BRCA1/2 cells (72, 73). Women with a heterozygous deleterious germline mutation of *BRCA1* or *BRCA2* carry an increased risk of developing breast or ovarian cancer (74). Since BRCAs are involved in HR and the dependence of DNA breaks by repair through PARP1, these cells are exquisitely sensitive to PARP inhibition. Cells in persons with an inherited *BRCA* mutation contain a wild-type and a mutated *BRCA* gene. Malignant cells, however, are homozygous with respect to the *BRCA* mutation status. HR remains intact in the presence of a normal *BRCA* allele, therefore non-malignant cells should be much less affected by PARP inhibition. The preclinical studies demonstrated a synthetic lethal interaction of PARP1 inhibition with *BRCA*1/2 mutation. In xenograft studies, pharmacological inhibition of PARP1 by AG14361 or KU0058684 resulted in tumor regression in BRCA1/2-deficient cell lines compared to BRCA1/2 wild-type or heterozygote cell lines (72, 73). Clinical proof-of-concept has been achieved with PARP inhibitor olaparib. Antitumor efficacy has only been observed with *BRCA*1/2 mutation carriers (75). Poly-ADP-formation and γH2X was assessed in peripheral blood mononuclear cells and plucked eyebrow-hair follicles and indicated that more than 90% PARP inhibition was achieved with tolerable doses. Phase II studies in chemotherapy-refractory breast and ovarian tumors with *BRCA*1/2 mutations indicated 41% and 33% objective response rates according to the response evaluation criteria in solid tumors (RECIST) (76, 77).

PARP inhibitors are also evaluated in patients with triple negative breast cancer (TNBC), an aggressive subtype of breast cancer that lacks expression of ER and PR and HER2 (78). BRCA-deficient basal-like tumors and TNBC show clinical and pathologic similarities (79-81). The BRCA pathway and subsequent HR is compromised in a subset of TNBC patients. The molecular basis is due to different mechanisms such as decreased *BRCA1* messenger RNA expression (82), *BRCA* gene promoter methylation, overexpression and copy number gain of *ID4*, a negative regulator of BRCA1 (83), and copy number aberrations affecting genes within the BRCA damage-response pathway. These observations support the concept of defective HR in patients with TNBC which is referred to as BRCAness (84). Preliminary results of evaluation of a number of PARP inhibitors in patients with BRCA-mutated tumors and TNBC are summarized in (85). RAD51 foci formation is explored as a biomarker for HR function and sensitivity to PARP inhibitors. Cells with intact HR form RAD51 foci within the nucleus due to the localization of RAD51 to double-stranded DNA breaks. These foci can be visualized by immunohistochemistry. Dysfunction of BRCA1/2 restricts formation of RAD51 foci (86).

MMR and SL

A synthetic lethal interaction between MSH2 deficiency and treatment with methotrexate has been identified (87). MSH2-deficient cells accumulate 8-oxo-guanine (8-oxoG) after treatment with methotrexate in contrast to MSH2-proficient cells. Accumulation of this DNA-damage product seems to be incompatible with cell survival. A corresponding phase II study is ongoing with incorporation of 8-oxoG as a biomarker. In extension of these findings it was shown that MSH2-deficient cells are killed by inhibition of DNA polymerase β (DNA Polβ), in contrast MLH1/DNA Polβ SL was not observed. Inhibition of DNA Polγ is synthetically lethal with MLH1 deficiency (88). Interestingly, MSH2/DNA Polβ SL correlated with nuclear accumulation of 8-oxoG DNA lesions, whereas in the case of MLH1/DNA Polγ SL, 8-oxoG DNA lesions accumulated in mitochondria pointing to distinct nuclear and mitochondrial pathways of MMR (89, 90). Current estimates indicate that defective MMR affects 15-17% of all colorectal carcinomas (91, 92). MMR deficiency might result from inheritance of a mutated MMR gene, a somatic mutation of a MMR gene, epigenetic modification of MMR genes or a combination of mutation and epigenetic modification. Affected genes are *MLH1*, *MSH2*, *MSH6* and *PMS2* (93, 94). Recently it was shown that silencing of PTEN-induced putative kinase 1 (*PINK1*) is synthetically lethal in MMR deficient cell lines based on dysfunction of MSH2, MLH1 or MSH6 (95). Inhibition of PINK1 resulted in increase of reactive oxygen species and nuclear and mitochondrial oxidative lesions based on 8-oxoG. Increase of 8-oxoG lesions holds true for SL based on MSH2/Polβ, MLH1/Polγ and PINK1/MMR deficiency, indicating that quantitation of 8-oxoG lesions might be a potential drug efficacy marker. Loss of PTEN function has been shown to result in SL together with PARP inhibition (96). Cells with PTEN deficiency were 20-fold more sensitive to PARP inhibitors. *PTEN* null cells showed defective checkpoint regulation after treatment with ionizing irradiation resulting in aneuploidy (97) and chromosomal instability in *PTEN* null cells due to decreased levels of RAD51, which is involved in double-stranded break repair (98). Interestingly, the effect of PTEN in this context was not dependent on its phosphatase activity, but was at least partially dependent on its ability to shuttle to the nucleus.

Contextual SL

Hypoxia can inhibit DNA MMR, NER and double-strand break repair (DBR) (99). Functional heterozygosity of repair-related genes can occur through mutation, deletion or hypermethylation. Hypoxia can lead to decreased expression of the remaining allele of repair-related genes and therefore contribute to tumor progression, but also render hypoxic cells susceptible to SL. Reduced expression of genes involved in HR such as *RAD51, BRCA1* and *BRCA2* under hypoxic conditions has been reported (100, 101). PARP inhibitors might confer SL in this context with the caveat that proliferation of tumor cells is required because PARP inhibitors confer their toxicity by inducing collapsed replication forks (73). It has been shown that reductions in DNA-repair gene expression by moderate levels of hypoxia is still compatible with proliferation (99). Recently it was shown that under acute or prolonged hypoxia, survival of tumor cells was dramatically decreased after treatment with PARP inhibitors, including a decrease in RAD51 (102). Irradiated tumors that had been pre-treated with PARP inhibitors had a lower survival time than control xenografts that were treated with vehicle alone. Notably, non-tumor cells were not killed by the combination of irradiation and PARP inhibitors. The issues as outlined have to be investigated in more detail, especially in the context of imaging techniques for monitoring tumor hypoxia and responsiveness to inhibitors of repair-related pathways.

Conclusion

As already outlined, treatment of tumors addicted to activated pathways can result in significant improvement in terms of progression-free survival and overall survival. Oncogene addiction due to activating point mutations or fusion proteins is an Achilles' heel of defined subtypes of cancer regarding treatment with anti-cancer therapeutics. Patients with corresponding alterations can be identified with appropriate diagnostic tests resulting in identification of a subpopulation of patients with potential sensitivity for the corresponding drugs. Another Achilles' heel of tumor cells is based on vulnerability of tumor subtypes with respect to simultaneous inactivation of two genes such as inactivation of a tumor suppressor gene and another defined gene, inhibition of a mutated or overexpressed oncogene together with another gene, or inactivation of two defined genes which are part of a protein kinase network. Inactivation status of tumor suppressor genes, mutation status, overexpression of oncogenes and activation status of protein kinases under consideration can be determined in tumor biopsies with diagnostic tests depending on the targets under consideration. Sequencing of the genome of defined tumor entities in combination with RNAi-based screens for synthetic lethality will probably identify new targets for personalized treatment of cancer.

As outlined many clinical studies covering DNA-repair related SL are ongoing and a clearer picture between the usefulness of biomarkers, clinical efficacy and correlation with defined target-related molecular alterations will emerge. Clinical proof-of-concept has been demonstrated by inhibition of PARP in BRCA1/2-deficient breast and ovarian tumors and inhibition of mTOR in renal carcinomas with *VHL* mutations. Clinical studies targeting other types of SL will soon be started. Also the potential development of resistance mechanisms will be a matter of investigation. Reconstitution of BRCA function has been observed after treatment of BRCA-deficient (point mutated) breast cancer cells with a PARP inhibitor (103). Restoration of *p53* and *VHL* tumor suppressor gene function based on mutations after treatment with a corresponding inhibitor are also formally possible, but have not been reported. Substitution of protein kinase-related SL interactions by activation of other pathways or the emergence of activating mutations in the case of targeting enzymes are also possible alerting issues. In any case, SL-based approaches for treatment of cancer will extend the concept of personalized treatment of cancer.

References

1 Lord CJ and Ashworth A: Biology-driven cancer drug development: back to the future. BMC Biol *8*: 38, 2010.

2 Melo JV and Deininger MW: Biology of chronic myelogenous leukemia – signaling pathways of initiation and transformation. Hematol Oncol Clin North Am *18*: 545-568, 2004.

3 Zhang J, Yang PL and Gray NS: Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer *9*: 28-39, 2009.

4 Gerber DE and Minna JD: ALK inhibition for non-small cell lung cancer: from discovery to therapy in record time. Cancer Cell *18*: 548-551, 2010.

5 Tucker CL and Fields S: Lethal combinations. Nat Genet *35*: 204-205, 2003.

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

7 Simon JA, Szankasi P, Nguyen DK, Ludlow C, Dunstan HM, Roberts CJ, Jensen EL and Hartwell LH and Friend SH: Differential toxicities of anticancer agents among DNA repair and checkpoint mutants of *Saccharomyces cerevisiae*. Cancer Res *60*: 328-333, 2000.

8 Kaelin WG Jr.: The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Cancer *5*: 689-698, 2005.

9 Kaelin WG Jr.: Synthetic lethality: a framework for the development of wiser cancer therapeutics. Genome Med *1*: 99, 2009.

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

11 Mizuarai S and Kotani H: Synthetic lethal interactions for the development of cancer therapeutics: biological and methodological advancements. Hum Genet *128*: 567-575, 2010.

12 Scholl C, Fröhling S, Dunn IF, Schinzel AC, Barbie DA, Kim SY, Silver SJ, Tamayo P, Wadlow RC, Ramaswamy S, Döhner K, Bullinger L, Sandy P, Boehm JS, Root DE, Jacks T, Hahn WC and Gilliland DG: Synthetic lethal interaction between oncogenic *KRAS* dependency and *STK33* suppression in human cancer cells. Cell *137*: 821-834, 2009.

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

14 Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E, Scholl C, Fröhling S, Chan EM, Sos ML, Michel K, Mermel C, Silver SJ, Weir BA, Reiling JH, Sheng Q, Gupta PB, Wadlow RC, Le H, Hoersch S, Wittner BS, Ramaswamy S, Livingston DM, Sabatini DM, Meyerson M, Thomas RK, Lander ES, Mesirov JP, Root DE, Gilliland DG, Jacks T and Hahn WC: Systematic RNA interference reveals that oncogenic *KRAS*-driven cancers require TBK1. Nature *462*: 108-112, 2009.

15 Puyol M, Martín A, Dubus P, Mulero F, Pizcueta P, Khan G, Guerra C, Santamaría D and Barbacid M: 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, 2010.

16 Zhu T, Chen L, Du W, Tsuji T and Chen C: Synthetic lethality induced by loss of PKC δ and mutated Ras. Genes Cancer *1*: 142-151, 2010.

17 Torrance CJ, Agrawal V, Vogelstein B and Kinzler KW: Use of isogenic human cancer cells for high-throughput screening and drug discovery. Nat Biotechnol *19*: 940-945, 2001.

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

19 Downward J: Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer *3*: 11-22, 2003.

20 Maslon MM and Hupp TR: Drug discovery and mutant p53. Trends Cell Biol *20*: 542-555, 2010.

21 Brosh R and Rotter V: When mutants gain new powers: news from the mutant p53 field. Nat Rev Cancer *9*: 701-713, 2009.

22 Staples OD, Steele RJ and Lain S: p53 as a therapeutic target. Surgeon *6*: 240-243, 2008.

23 Weinstein JN, Myers TG, O'Connor PM, Friend SH, Fornace AJ Jr, Kohn KW, Fojo T, Bates SE, Rubinstein LV, Anderson NL, Buolamwini JK, van Osdol WW, Monks AP, Scudiero DA, Sausville EA, Zaharevitz DW, Bunow B, Viswanadhan VN, Johnson GS, Wittes RE and Paull KD: An information-intensive approach to the molecular pharmacology of cancer. Science *275*: 343-349, 1997.

24 Zhang CC, Yang JM, Bash-Babula J, White E, Murphy M, Levine AJ and Hait WN: DNA damage increases sensitivity to vinca alkaloids and decreases sensitivity to taxanes through p53-dependent repression of microtubule-associated protein 4. Cancer Res *59*: 3663-3670, 1999.

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

26 Robinson M, Jiang P, Cui J, Li J, Wang Y, Swaroop M, Madore S, Lawrence TS and Sun Y: Global genechip profiling to identify genes responsive to p53-induced growth arrest and apoptosis in human lung carcinoma cells. Cancer Biol Ther *2*: 406-415, 2003.

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

28 Wang Q, Fan S, Eastman A, Worland PJ, Sausville EA and O'Connor PM: UCN-01: a potent abrogator of G_2 checkpoint function in cancer cells with disrupted p53. J Natl Cancer Inst *88*: 956-965, 1996.

29 Rowley R, Hudson J and Young PG: The wee1 protein kinase is required for radiation-induced mitotic delay. Nature *356*: 353-355, 1992.

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

31 Wang Y, Decker SJ and Sebolt-Leopold J: Knockdown of Chk1, Wee1 and Myt1 by RNA interference abrogates G_2 checkpoint and induces apoptosis. Cancer Biol Ther *3*: 305-313, 2004.

32 Jiang H, Reinhardt HC, Bartkova J, Tommiska J, Blomqvist C, Nevanlinna H, Bartek J, Yaffe MB and Hemann MT: The combined status of ATM and p53 link tumor development with therapeutic response. Genes Dev *23*: 1895-1909, 2009.

33 Mizuarai S, Yamanaka K and Kotani H: Mutant p53 induces the *GEF-H1* oncogene, a guanine nucleotide exchange factor-H1 for RhoA, resulting in accelerated cell proliferation in tumor cells. Cancer Res *66*: 6319-6326, 2006.

34 Baldwin A, Grueneberg DA, Hellner K, Sawyer J, Grace M, Li W, Harlow E and Munger K: Kinase requirements in human cells: V. Synthetic lethal interactions between p53 and the protein kinases SGK2 and PAK3. Proc Natl Acad Sci USA *107*: 12463-12468, 2010.

35 Kumar R, Gururaj AE and Barnes CJ: p21-activated kinases in cancer. Nat Rev Cancer *6*: 459-471, 2006.

36 Reinhardt HC, Aslanian AS, Lees JA and Yaffe MB: p53-deficient cells rely on ATM- and ATR-mediated checkpoint signaling through the p38MAPK/MK2 pathway for survival after DNA damage. Cancer Cell *11*: 175-189, 2007.

37 Bauzon F and Zhu L: Racing to block tumorigenesis after pRb loss: An innocuous point mutation wins with synthetic lethality. Cell Cycle *9*: 2118-2123, 2010.

38 Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, Petersen R, Frost P, Gibbons JJ, Wu H and Sawyers CL: Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. Proc Natl Acad Sci USA *98*: 10314-10419, 2001.

39 Dong Y, Li A, Wang J, Weber JD and Michel LS: Synthetic lethality through combined Notch-epidermal growth factor receptor pathway inhibition in basal-like breast cancer. Cancer Res *70*: 5465-5474, 2010.

40 Michiue H, Eguchi A, Scadeng M and Dowdy SF: Induction of *in vivo* synthetic lethal RNAi responses to treat glioblastoma. Cancer Biol Ther *8*: 2306-2313, 2009.

41 Hulleman E and Helin K: Molecular mechanisms in gliomagenesis. Adv Cancer Res *94*: 1-27, 2005.

42 Potratz JC, Saunders DN, Wai DH, Ng TL, McKinney SE, Carboni JM, Gottardis MM, Triche TJ, Jürgens H, Pollak MN, Aparicio SA and Sorensen PH: Synthetic lethality screens

reveal RPS6 and MST1R as modifiers of insulin-like growth factor-1 receptor inhibitor activity in childhood sarcomas. Cancer Res *70*: 8770-8781, 2010.

43 Meyer N and Penn LZ: Reflecting on 25 years with *MYC*. Nat Rev Cancer *8*: 976-990, 2008.

44 Pelengaris S, Khan M and Evan G: *c-MYC*: more than just a matter of life and death. Nat Rev Cancer *2*: 764-776, 2002.

45 Clausen DM, Guo J, Parise RA, Beumer JH, Egorin MJ, Lazo JS, Prochownik EV and Eiseman JL: *In vitro* cytotoxicity and *in vivo* efficacy, pharmacokinetics, and metabolism of 10074-G5, a novel small-molecule inhibitor of c-Myc/Max dimerization. J Pharmacol Exp Ther *335*: 715-727, 2010.

46 Maclean KH, Dorsey FC, Cleveland JL and Kastan MB: Targeting lysosomal degradation induces p53-dependent cell death and prevents cancer in mouse models of lymphomagenesis. J Clin Invest *118*: 79-88, 2008.

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

48 Rottmann S, Wang Y, Nasoff M, Deveraux QL and Quon KC: A TRAIL receptor-dependent synthetic lethal relationship between MYC activation and GSK3beta/FBW7 loss of function. Proc Natl Acad Sci USA *102*: 15195-21500, 2005.

49 Bhatia K, Huppi K, Spangler G, Siwarski D, Iyer R and Magrath I: Point mutations in the *c-Myc* transactivation domain are common in Burkitt's lymphoma and mouse plasmacytomas. Nat Genet *5*: 56-61, 1993.

50 Gregory MA and Hann SR: c-Myc proteolysis by the ubiquitin-proteasome pathway: stabilization of c-Myc in Burkitt's lymphoma cells. Mol Cell Biol *20*: 2423-2435, 2000.

51 Welcker M and Clurman BE: FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. Nat Rev Cancer *8*: 83-93, 2008.

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

53 Molenaar JJ, Ebus ME, Geerts D, Koster J, Lamers F, Valentijn LJ, Westerhout EM, Versteeg R and Caron HN: Inactivation of CDK2 is synthetically lethal to MYCN over-expressing cancer cells. Proc Natl Acad Sci USA *106*: 12968-12973, 2009.

54 Yang D, Liu H, Goga A, Kim S, Yuneva M and Bishop JM: 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, 2010.

55 den Hollander J, Rimpi S, Doherty JR, Rudelius M, Buck A, Hoellein A, Kremer M, Graf N, Scheerer M, Hall MA, Goga A, von Bubnoff N, Duyster J, Peschel C, Cleveland JL, Nilsson JA and Keller U: Aurora kinases A and B are up-regulated by Myc and are essential for maintenance of the malignant state. Blood *116*: 1498-1505, 2010.

56 Kaelin WG: Von Hippel-Lindau disease. Annu Rev Pathol *2*: 145-173, 2007.

57 Kaelin WG Jr and Maher ER: The *VHL* tumour-suppressor gene paradigm. Trends Genet *14*: 423-426, 1998.

58 Sutphin PD, Chan DA, Li JM, Turcotte S, Krieg AJ and Giaccia AJ: Targeting the loss of the von Hippel-Lindau tumor suppressor gene in renal cell carcinoma cells. Cancer Res *67*: 5896-5905, 2007.

59 Turcotte S, Sutphin PD and Giaccia AJ: Targeted therapy for the loss of von Hippel-Lindau in renal cell carcinoma: a novel molecule that induces autophagic cell death. Autophagy *4*: 944-946, 2008.

60 Chan DA and Giaccia AJ: Targeting cancer cells by synthetic lethality: autophagy and *VHL* in cancer therapeutics. Cell Cycle *7*: 2987-2990, 2008.

61 Thomas GV, Tran C, Mellinghoff IK, Welsbie DS, Chan E, Fueger B, Czernin J and Sawyers CL: Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. Nat Med *12*: 122-127, 2006.

62 Bommi-Reddy A, Almeciga I, Sawyer J, Geisen C, Li W, Harlow E, Kaelin WG Jr and Grueneberg DA: Kinase requirements in human cells: III. Altered kinase requirements in *VHL*$^{-/-}$ cancer cells detected in a pilot synthetic lethal screen. Proc Natl Acad Sci USA *105*: 16484-16489, 2008.

63 Annunziata CM and O'Shaughnessy J: Poly (ADP-ribose) polymerase as a novel therapeutic target in cancer. Clin Cancer Res *16*: 4517-4526, 2010.

64 Helleday T, Petermann E, Lundin C, Hodgson B and Sharma RA: DNA repair pathways as targets for cancer therapy. Nat Rev Cancer *8*: 193-204, 2008.

65 Tutt A and Ashworth A: The relationship between the roles of *BRCA* genes in DNA repair and cancer predisposition. Trends Mol Med *8*: 571-576, 2002.

66 Wu L: Wrestling off RAD51: a novel role for RecQ helicases. Bioessays *30*: 291-295, 2008.

67 Lord CJ and Ashworth A: Targeted therapy for cancer using PARP inhibitors. Curr Opin Pharmacol *8*: 363-369, 2008.

68 Rouleau M, Patel A, Hendzel MJ, Kaufmann SH and Poirier GG: PARP inhibition: PARP1 and beyond. Nat Rev Cancer *10*: 293-301, 2010.

69 Shrivastav M, De Haro LP and Nickoloff JA: Regulation of DNA double-strand break repair pathway choice. Cell Res *18*: 134-147, 2008.

70 Martin SA, Lord CJ and Ashworth A: Therapeutic targeting of the DNA mismatch repair pathway. Clin Cancer Res *16*: 5107-5113, 2010.

71 Jiricny J: The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol *7*: 335-346, 2006.

72 Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, Martin NM, Jackson SP, Smith GC and Ashworth A: Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy. Nature *434*: 917-921, 2005.

73 Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ and Helleday T: Specific killing of *BRCA2*-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature *434*: 913-917, 2005.

74 Chen S and Parmigiani G: Meta-analysis of *BRCA1* and *BRCA2* penetrance. J Clin Oncol *25*: 1329-1333, 2007.

75 Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, Ashworth A, Carmichael J, Kaye SB, Schellens JH and de Bono JS: Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers. N Engl J Med *361*: 123-134, 2009.

76 Audeh MW, Carmichael J, Penson RT, Friedlander M, Powell B, Bell-McGuinn KM, Scott C, Weitzel JN, Oaknin A, Loman N, Lu K, Schmutzler RK, Matulonis U, Wickens M and Tutt A: Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with *BRCA1* or *BRCA2* mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet *376*: 245-251, 2010.

77 Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, Friedlander M, Arun B, Loman N, Schmutzler RK, Wardley A, Mitchell G, Earl H, Wickens M and Carmichael J: Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with *BRCA1* or *BRCA2* mutations and advanced breast cancer: a proof-of-concept trial. Lancet *376*: 235-244, 2010.

78 Anders CK, Winer EP, Ford JM, Dent R, Silver DP, Sledge GW and Carey LA: Poly(ADP-Ribose) polymerase inhibition: 'Targeted' therapy for triple-negative breast cancer. Clin Cancer Res *16*: 4702-4710, 2010.

79 Lark AL, Livasy CA, Dressler L, Moore DT, Millikan RC, Geradts J, Iacocca M, Cowan D, Little D, Craven RJ and Cance W: High focal adhesion kinase expression in invasive breast carcinomas is associated with an aggressive phenotype. Mod Pathol *18*: 1289-1294, 2005.

80 Cleator S, Heller W and Coombes RC: Triple-negative breast cancer: therapeutic options. Lancet Oncol *8*: 235-244, 2007.

81 Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein Lønning P and Børresen-Dale AL: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA *98*: 10869-10874, 2001.

82 Natrajan R, Weigelt B, Mackay A, Geyer FC, Grigoriadis A, Tan DS, Jones C, Lord CJ, Vatcheva R, Rodriguez-Pinilla SM, Palacios J, Ashworth A and Reis-Filho JS: An integrative genomic and transcriptomic analysis reveals molecular pathways and networks regulated by copy number aberrations in basal-like, HER2 and luminal cancers. Breast Cancer Res Treat *121*: 575-589, 2010.

83 Turner N, Tutt A and Ashworth A: Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer *4*: 814-819, 2004.

84 Rehman FL, Lord CJ and Ashworth A: Synthetic lethal approaches to breast cancer therapy. Nat Rev Clin Oncol *7*: 718-724, 2010.

85 Graeser M, McCarthy A, Lord CJ, Savage K, Hills M, Salter J, Orr N, Parton M, Smith IE, Reis-Filho JS, Dowsett M, Ashworth A and Turner NC: A marker of homologous recombination predicts pathologic complete response to neoadjuvant chemotherapy in primary breast cancer. Clin Cancer Res *16*: 6159-6168, 2010.

86 Mukhopadhyay A, Elattar A, Cerbinskaite A, Wilkinson SJ, Drew Y, Kyle S, Los G, Hostomsky Z, Edmondson RJ and Curtin NJ: Development of a functional assay for homologous recombination status in primary cultures of epithelial ovarian tumor and correlation with sensitivity to poly(ADP-ribose) polymerase inhibitors. Clin Cancer Res *16*: 2344-2351, 2010.

87 Martin SA, McCarthy A, Barber LJ, Burgess DJ, Parry S, Lord CJ and Ashworth A: Methotrexate induces oxidative DNA damage and is selectively lethal to tumour cells with defects in the DNA mismatch repair gene *MSH2*. EMBO Mol Med *1*: 323-337, 2009.

88 Martin SA, McCabe N, Mullarkey M, Cummins R, Burgess DJ, Nakabeppu Y, Oka S, Kay E, Lord CJ and Ashworth A: DNA polymerases as potential therapeutic targets for cancers deficient in the DNA mismatch repair proteins *MSH2* or *MLH1*. Cancer Cell *17*: 235-248, 2010.

89 de Souza-Pinto NC, Mason PA, Hashiguchi K, Weissman L, Tian J, Guay D, Lebel M, Stevnsner TV, Rasmussen LJ and Bohr VA: Novel DNA mismatch-repair activity involving YB-1 in human mitochondria. DNA Repair *8*: 704-719, 2009.

90 Duval A and Hamelin R: Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability. Cancer Res *62*: 2447-2454, 2002.

91 Imai K and Yamamoto H: Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. Carcinogenesis *29*: 673-680, 2008.

92 Popat S, Hubner R and Houlston RS: Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol *23*: 609-618, 2005.

93 Hewish M, Lord CJ, Martin SA, Cunningham D and Ashworth A: Mismatch repair deficient colorectal cancer in the era of personalized treatment. Nat Rev Clin Oncol *7*: 197-208, 2010.

94 Lynch HT, Lynch JF, Lynch PM and Attard T: Hereditary colorectal cancer syndromes: molecular genetics, genetic counseling, diagnosis and management. Fam Cancer *7*: 27-39, 2008.

95 Martin SA, Hewish M, Sims D, Lord CJ and Asworth A: Parallel high-throughput RNA interference screens identify PINK1 as a potential therapeutic target for the treatment of DNA mismatch repair-deficient cancers. Cancer Res *71*: 1836-1848, 2011.

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

97 Puc J, Keniry M, Li HS, Pandita TK, Choudhury AD, Memeo L, Mansukhani M, Murty VV, Gaciong Z, Meek SE, Piwnica-Worms H, Hibshoosh H and Parsons R: Lack of *PTEN* sequesters CHK1 and initiates genetic instability. Cancer Cell *7*: 193-204, 2005.

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

99 Chan N, Pires IM, Bencokova Z, Coackley C, Luoto KR, Bhogal N, Lakshman M, Gottipati P, Oliver FJ, Helleday T, Hammond EM and Bristow RG: Contextual synthetic lethality of cancer cell kill based on the tumor microenvironment. Cancer Res *70*: 8045-8054, 2010.

100 Bindra RS, Gibson SL, Meng A, Westermark U, Jasin M, Pierce AJ, Bristow RG, Classon MK and Glazer PM: Hypoxia-induced down-regulation of *BRCA1* expression by E2Fs. Cancer Res *65*: 11597-11604, 2005.

101 Chan N, Koritzinsky M, Zhao H, Bindra R, Glazer PM, Powell S, Belmaaza A, Wouters B and Bristow RG: Chronic hypoxia decreases synthesis of homologous recombination proteins to offset chemoresistance and radioresistance. Cancer Res *68*: 605-614, 2008.

102 Meng AX, Jalali F, Cuddihy A, Chan N, Bindra RS, Glazer PM and Bristow RG: Hypoxia down-regulates DNA double-strand break repair gene expression in prostate cancer cells. Radiother Oncol *76*: 168-176, 2005.

103 Ashworth A: Drug resistance caused by reversion mutation. Cancer Res *68*: 10021-10023, 2008.

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