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Inhibition of the Replication Stress Response Is a Synthetic Vulnerability in SCLC That Acts Synergistically in Combination with Cisplatin

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Abstract

Small cell lung cancer (SCLC) is generally regarded as very difficult to treat, mostly due to the development of metastases early in the disease and a quick relapse with resistant disease. SCLC patients initially show a good response to treatment with the DNA damaging agents cisplatin and etoposide. This is, however, quickly followed by the development of resistant disease, which urges the development of novel therapies for this type of cancer. In this study, we set out to compile a comprehensive overview of the vulnerabilities of SCLC. A functional genome-wide screen where all individual genes were knocked out was performed to identify novel vulnerabilities of SCLC. By analysis of the knockouts that were lethal to these cancer cells, we identified several processes to be synthetic vulnerabilities in SCLC. We were able to validate the vulnerability to inhibition of the replication stress response machinery by use of Chk1 and ATR inhibitors. Strikingly, SCLC cells were more sensitive to these inhibitors than nontransformed cells. In addition, these inhibitors work synergistically with either etoposide and cisplatin, where the interaction is largest with the latter. ATR inhibition by VE-822 treatment in combination with cisplatin also outperforms the combination of cisplatin with etoposide *in vivo*. Altogether, our study uncovered a critical dependence of SCLC on the replication stress response and urges the validation of ATR inhibitors in combination with cisplatin in a clinical setting.

Introduction

Small cell lung cancer (SCLC) comprises around 20% of all lung cancer cases, and with a dismal 5-year survival rate of only 5%, it is regarded the most aggressive type of lung cancer (1). As SCLC disseminates already early in disease development, surgery is generally not applicable and treatment therefore relies on the systemic delivery of chemotherapeutics. Given that this type of tumor is highly responsive to DNA damaging agents, a combination of cisplatin and etoposide was put forward as the standard of care (2). Even though most SCLC cases initially show a good response to this combination, this is almost uniformly followed by a relapse with resistant disease (3). The only available second-line treatment, topotecan, shows a low response rate of only 20% (4, 5). As treatment options for SCLC are currently limited, novel therapies are urgently awaited.

In stark contrast with other tumor types, the introduction of targeted drugs has not brought novel therapeutic approaches for SCLC to the clinic. This mainly stems from the fact that this type of tumor lacks available druggable targets. There are some exceptions, where for example *FGFR1* is found amplified in around 5% to 10% of SCLC cases, which could be targeted by FGFR inhibitors (6, 7). Other than that, the most commonly found alterations in this type of tumor are loss of *TP53* and *RB1*, and amplification and/or overexpression of *MYC*-family members and *NFIB* (8–11). Even though these alterations are not directly druggable themselves, they do lead to alterations in several cellular non-oncogenic processes. In addition, they generate altered dependencies on several stress responses that nontransformed cells do not have. It has been demonstrated that many cancer cells critically rely on these stress responses for their survival, and hence these have been dubbed synthetic vulnerabilities or non-oncogene addictions (12). Because SCLC lacks available actionable targets, targeting SCLC-specific synthetic vulnerabilities would be an alternative approach to combat this type of cancer.

In the current study, we performed a genome-wide CRISPR-Cas9 screen to identify novel targets in SCLC cells. With this approach, it was shown that the replication stress response (RSS) is a potent synthetic vulnerability in this tumor type. Inhibition of the RSS kinases ATR or CHK1 showed high efficacy in SCLC, which likely depends on high expression of *MYC*-family members. In addition, a large synergy between ATR inhibition and cisplatin was observed and a combination of these compounds exceeded the effects of the current standard of care in our preclinical models.

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Materials and Methods

Cell lines

Human cell lines DMS-273, NCI-H446, NCI-H1304, NCI-H69, NCI-H82, NCI-H187, SW1573, A549, NCI-H226, NCI-H522, SK-MES-1, and H1299 were obtained from the American Type Culture Collection. BJ primary fibroblasts were a kind gift from Prof. Reuven Agami (The Netherlands Cancer Institute, Amsterdam); primary mouse embryonic fibroblasts (MEF) were isolated as previously described (13). All these cell lines were cultured in DMEM/F12 supplemented with 10% fetal calf serum and 100 units/mL penicillin and 100 μg/mL streptomycin or in RPMI supplemented with 10% fetal calf serum, 1 mmol/L sodium pyruvate, and 100 units/mL penicillin and 100 μg/mL streptomycin (all Gibco). LMYC5 mouse SCLC cells were previously generated from a $TP53^{-/-}$ $RB1^{-/-}$ LMYC mouse model (14). These cells were grown in DMEM/F12 supplemented with 10% fetal calf serum, HITES (Gibco), and penicillin/streptomycin. All cells were grown in a humidified atmosphere of 5% CO2 at 37°C.

Genome-wide CRISPR screen

LMYC5 cells were transduced with lentiviral particles produced from pGS-CAS9 (gifted by Stefano Annunziato), after which cells were selected with Neomycin. Next, cells were transduced with a high titer library of lentiviruses containing ~90,000 unique gRNAs using an MOI of 0.3, and cells were selected using puromycin (Addgene; ref. 15). The library was maintained at a 500× complexity. Cells were split in three triplicates and cultured for 14 days, after which the cells were harvested and genomic DNA was isolated using a phenol-chloroform extraction. Next, two rounds of PCRs were performed with the primers indicated in Supplementary Table S1 to amplify the sgRNA inserts. The sgRNA abundance was determined by next-generation sequencing. Differential sgRNA abundance between $t = 0$ and $t = 14$ days was determined using the MaGeCK algorithm (16). GO term analysis was performed using Ingenuity Pathway Analysis software (Qiagen).

Dose response curves

Dose response curves were generated using the indicated cell lines with optimal seeding densities in 96-well plates. Cells were treated with 2-fold serial dilutions of the compounds. Compounds used were etoposide, VE-822, AZD7762 (dissolved in DMSO, Selleckchem; refs. 17, 18), and cisplatin (1 mg/mL in saline, Pharmachemie B.V.). Six days after addition of the compounds, cell viability was determined by reading out the fluorescent signal that was obtained after 2 hours of incubation with AlamarBlue (Invitrogen) using an Infinite M200 Pro reader (Tecan) as previously described (19). Synergy scores and volumes were calculated and plotted using MacSynergyII; ref. 20).

Drug response analyses

Gene expression and drug response data from Polley and colleagues (21) were downloaded from https://sclccelllines.cancer.gov/sclc/downloads.xhtml. We defined MYC-high and MYC-low groups by setting thresholds on MYC and MYCL1 expression. These thresholds were set at roughly half-way the range of the expression, in a low-density region of the distribution, following the mutual exclusive pattern of expression of the two genes. The MYC-high group was hereby defined as (MYC>8.5 or MYCL>9.5). For all CHK1 and ATR inhibitors in the data (22–26), we tested for a significant association between the MYC-high/MYC-low groups and the IC_{50} using a Mann–Whitney *U* test. The resulting *P* values were corrected for multiple testing using Benjamini–Hochberg.

In vivo treatment

One million DMS-273 or 2 million NCI-H187 cells in 1:1 GelTrex:PBS solution (Life Technologies) were injected in one flank of BALB/c nude mice. Treatment was started when tumor nodules reached 40 mm^3. VE-822 (30 mg/kg) was administered by oral gavage on 4 consecutive days each week. This compound was dissolved in 10% Vitamin E d-alpha tocopheryl polyethylene glycol 1000 succinate (Sigma). Cisplatin (3 mg/kg) was administered by intraperitoneal (i.p.) injection each week starting on the third day after tumors reached 40 mm^3. Etoposide (8 mg/kg) was administered i.p. 3 days each week. Tumor sizes were measured three times per week. The study was performed in accordance with the Dutch and European regulations on care and protection of laboratory animals. Mice were housed under standard conditions of feeding, light, and temperature with free access to food and water. All animal experiments were approved by the local animal experimental committee, DEC NKI.

Results

To identify novel druggable targets for the treatment of SCLC, we performed a genome-wide CRISPR-Cas9 screen in a cell line derived from a *Tp53*$^{-/-}$, *Rb1*$^{-/-}$, *Mycl1* genetically engineered SCLC mouse model (14). This cell line harbors the three most commonly found genetic alterations in SCLC, thereby making it a suitable model to identify novel synthetic vulnerabilities that depend on these genetic alterations. First, the cell line was transduced with Cas9 encoding lentiviral particles, after which the functionality of this protein was tested by introduction of unique sgRNAs and subsequent sequencing of the genomic loci targeted by these individual sgRNAs. As this test indicated that the Cas9 protein was functionally expressed and efficient in generating indels (Supplementary Fig. S1), the cell line was transduced with a lentiviral library containing approximately 90,000 unique sgRNAs targeting 19,500 genes with an average of four unique sgRNAs per gene. The cells were grown in three independent replicates for 2 weeks, after which the abundance of each sgRNA insert in the population was determined by next-generation sequencing. Comparison of the abundance of each sgRNA insert at the start of the experiment and after 2 weeks of growth showed a high correlation between all three replicates isolated from the same time point, indicating the high reproducibility of the screen (Supplementary Fig. S2). A differential analysis between the populations at the start and the end of the experiment showed that sgRNAs targeting 1,259 genes were significantly depleted from the population of cells that had been growing for 14 days (Fig. 1A; Supplementary Tables S2 and S3, FDR < 0.05). Among these were sgRNAs targeting *Mycl1* and *Ascl1*, two genes that are known to be essential for the survival of SCLC cells (27, 28). It is noteworthy that sgRNAs targeting *Tp53* and *Rb1* did not show any significant change in the screen, which is expected as these genes are deleted in the cell line used.

To find pathways essential for SCLC survival, a GO term enrichment analysis was performed on the identified genes. This

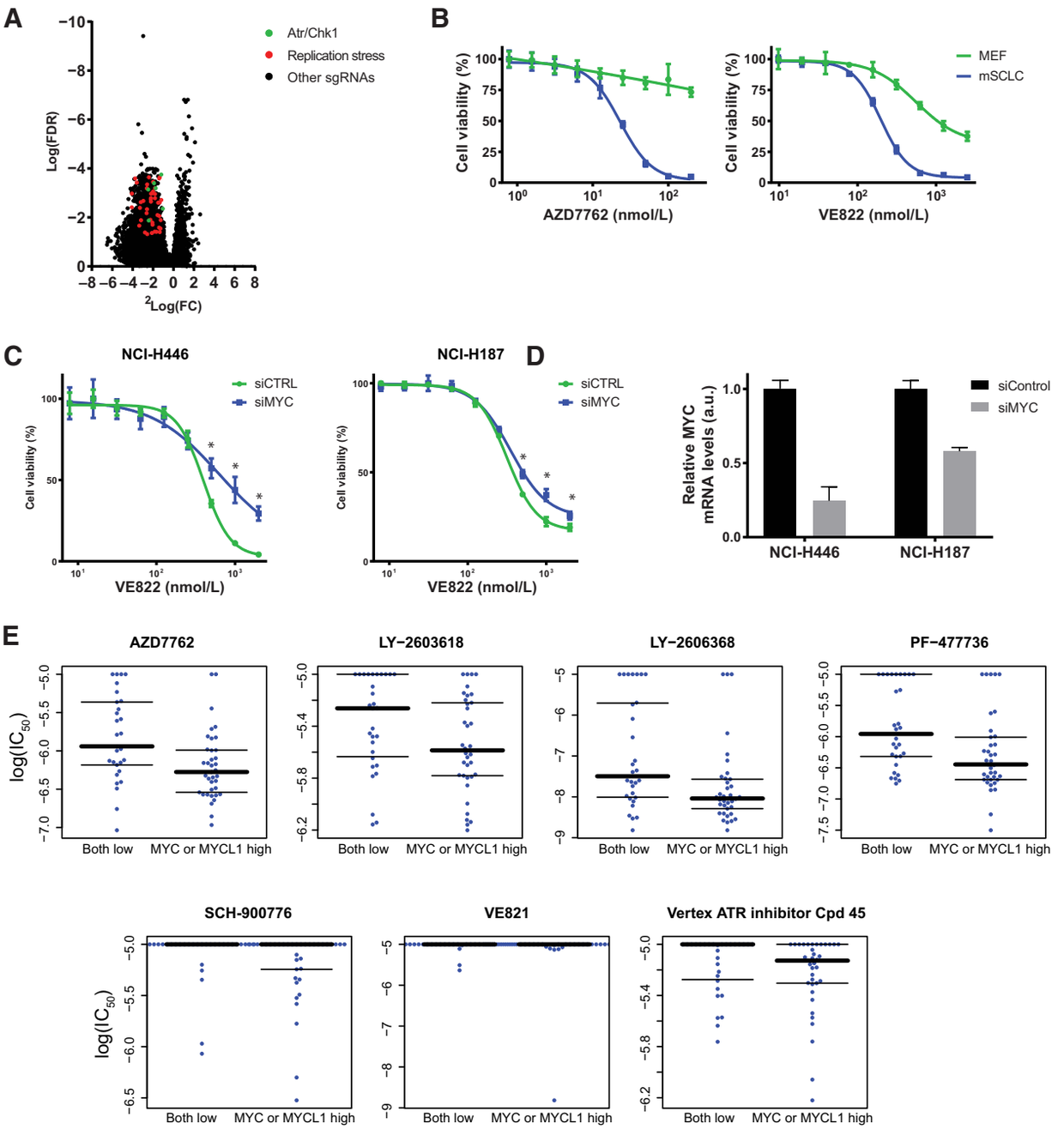

Figure 1.

Replication stress inhibition is a synthetic vulnerability in SCLC. **A,** Graph representing results from a genome-wide CRISPR-Cas9 screen for synthetic vulnerabilities in SCLC. The screen was performed in triplicate. Each dot represents the effect of a unique sgRNA. The sgRNAs targeting Atr and Chk1 are indicated in green, sgRNAs targeting genes that function in the RSS are indicated in red. **B,** Dose response curves of mSCLC and primary MEFs exposed to the CHK1 inhibitor AZD7762 and the ATR inhibitor VE-822. Values represent the mean ± standard deviation from three independent experiments. **C,** Dose response curves from NCI-H446 and NCI-H187 cells with and without MYC knockdown. Values represent the mean ± standard deviation from three independent experiments. *, $P = 0.01$. **D,** Relative MYC mRNA levels of the cell lines used in **C**. **E,** Graphs showing the $^2\log(IC_{50})$ for a selection of CHK1 and ATR inhibitors of a panel of 63 human SCLC cell lines screened by Polley et al. (21). Cell lines were grouped on the basis of absence or presence of *MYC* or *MYCL1* expression. *P* values were calculated using a Mann–Whitney *U* test; CHK1 inhibitors: AZD7762 ($P = 0.01$), LY-2603618 ($P = 0.01$), LY-2606368 ($P = 0.04$), PF-477736 ($P = 0.01$), SCH-900776 ($P = 0.05$), and ATR inhibitors: VE-821 ($P = 0.29$) and Vertex ATR inhibitor Cpd 45 ($P = 0.11$).

Table 1. Enriched GO terms in the genome-wide CRISPR-Cas9 screen

Process	*P* value
tRNA charging	4.13×10^{-23}
Cell-cycle control of chromosomal replication	$\mathbf{4.23 \times 10^{-19}}$
Protein ubiquitination pathway	4.29×10^{-19}
Estrogen receptor signaling	2.47×10^{-17}
Assembly of RNA polymerase II complex	6.03×10^{-17}
Mitotic roles of polo-like kinases	8.77×10^{-14}
Hereditary breast cancer signaling	6.63×10^{-12}
Nucleotide excision repair pathway	5.74×10^{-11}
Purine nucleotides *de novo* biosynthesis II	1.30×10^{-10}
Oxidative phosphorylation	2.35×10^{-10}

analysis showed that many genes belonging to the essentialome were also found to be required for SCLC survival (Table 1; Supplementary Table S4; ref. 29). Many genes belonging to the processes of tRNA charging, control of chromosomal replication, protein ubiquitination, assembly of RNA PolII, purine biosynthesis, and oxidative phosphorylation were all expected to be essential for the survival of any cell. Strikingly, the enrichment in the GO terms "mitotic roles of Polo-like kinases" and "hereditary breast cancer signaling" suggests that ubiquitous cancer-specific vulnerabilities were also detected by the screen.

A more in-depth analysis of the individual genes per GO term showed that the term "Cell-cycle control of chromosomal replication" comprised many genes that function in the RSS (Supplementary Table S5). This might not be surprising as oncogenes, such as *Mycl1*, which is present in the SCLC model used, are known to induce replication stress. Because *MYC*-family members are found amplified and/or overexpressed in more than 60% of human SCLC cases, the inhibition of replication stress repair systems was selected as our prime candidate for validation.

To test whether inhibition of the RSS is a synthetic vulnerability in SCLC, we exposed a mouse SCLC (mSCLC) cell line and primary MEFs to inhibitors of the RSS kinases ATR and CHK1, which were found as dropouts as well in the CRISPR-Cas9 screen (Supplementary Tables S3 and S5). This experiment showed that mSCLC cells are much more sensitive to these compounds than MEFs (Fig. 1B), suggesting that high levels of *Mycl1* oncogene might induce sensitivity to ATR and CHK1 inhibition. To functionally validate this observation, *MYC* was knocked down by means of RNA interference. This showed that upon knockdown of *MYC* the sensitivity to ATR inhibition decreased significantly (Fig. 1C and D). It is interesting to note that the effects observed in NCI-H446 cells are larger than the effects in NCI-H187 cells, which corresponds to the less efficient knockdown of MYC (Fig. 1C and D). To substantiate this further, the relation between expression of *MYC*-family members and responsiveness to ATR and CHK1 was analyzed in a data set generated on 63 human SCLC (21). These data showed that mRNA levels of *MYC* and *MYCL1* are mutually exclusive, as had been previously reported (Supplementary Fig. S3A, $P = 0.00078$; refs. 30, 31). Next, we defined a group of cells with low or high *MYC*/*MYCL1* expression by setting the cutoffs indicated in Supplementary Fig. S3B. By analyzing the IC_{50} of ATR and CHK1 inhibitors, we could show that high *MYC*/*MYCL1* expression is associated with sensitivity to CHK1 inhibitors (Fig. 1E). It is important to note that high expression of either gene alone is associated significantly with sensitivity to ATR and CHK1 inhibitors (Supplementary Fig. S3C).

For the ATR inhibitors VE821 and Cpd 45, no significant difference in sensitivity between the two groups was observed (Fig. 1E). However, as the majority of cell lines tested for the sensitivity to these compounds have a high IC_{50}, this more likely points to an ineffective dose used in the assay and capping of the data rather than an absence of sensitivity in the *MYC*/*MYCL1*-high group. Of note, *MYCN* expression, which was found in only a small subset of the cell lines in the panel, did not show a significant correlation with sensitivity to RSS inhibition (N. Aben, personal communication). This suggests that this *MYC*-family member might have different effects on replication stress.

Because the combination of cisplatin and etoposide is clinically used as the standard of care for SCLC patients, and because these compounds also act during replication, we wondered whether there would be synergy when either of these agents is combined with an ATR or a CHK1 inhibitor. SCLC cells were exposed to a range of combinations of drugs, after which the drug interactions were quantified using the MacSynergy II algorithm (20). This algorithm analyzes the drug interactions by means of computing a Bliss score, which unveils potential antagonism or synergy. By computing a Bliss score for each point in the combination matrix, a three-dimensional graph can demonstrate the mode of interaction between two drugs, with the volume under the graph representing the degree of synergy (Fig. 2A; Supplementary Fig. S4). Whereas the combination of cisplatin and etoposide did not show any synergy in an mSCLC line, the combinations of ATR and CHK1 inhibitors with etoposide, but especially with cisplatin, showed high levels of synergism (Fig. 2B–D). Strikingly, this synergy could not be observed in primary MEFs (Fig. 2E), suggesting that this synergy likely depends on high levels of *Mycl1* expression.

As murine SCLC does not contain the full range of mutations that human SCLC harbors, we tested the synergism between cisplatin and the ATR inhibitor VE-822 in a panel of human cell lines. The choice for VE-822 instead of the CHK1 inhibitor was guided by the current lack of clinical data on ATR inhibition in SCLC, thereby increasing the clinical relevance of these experiments. When combined with cisplatin, the VE-822 showed a remarkably high level of synergy (Fig. 3A). Interestingly, this synergy could not be detected when using primary human fibroblasts, suggesting that this combination affects nontransformed cells to much lower levels (Fig. 3B). When comparing the level of synergy between SCLC and NSCLC, it was observed that SCLC are significantly more sensitized to this combination (Fig. 3C; Supplementary Fig. S5A; Supplementary Table S6). To control for the efficacy of VE-822, the levels of its downstream target for phosphorylation, CHK1, were checked by Western blotting. This showed a large decrease in phospho-CHK1 levels after VE-822 administration (Supplementary Fig. S5B).

As the combination of VE-822 and cisplatin proved to be highly synergistic *in vitro*, we set out to test the efficacy of these compounds *in vivo*. This combination greatly reduced tumor growth in mice xenografted with DMS-273 and NCI-H187 cells when compared with the single agents (Fig. 4A and C). More importantly, this combination significantly exceeded the efficacy of the combination of cisplatin and etoposide, thereby showing the high potency of this combination in an *in vivo* setting. In addition, whereas combining cisplatin and etoposide significantly extended the overall survival of only the mice

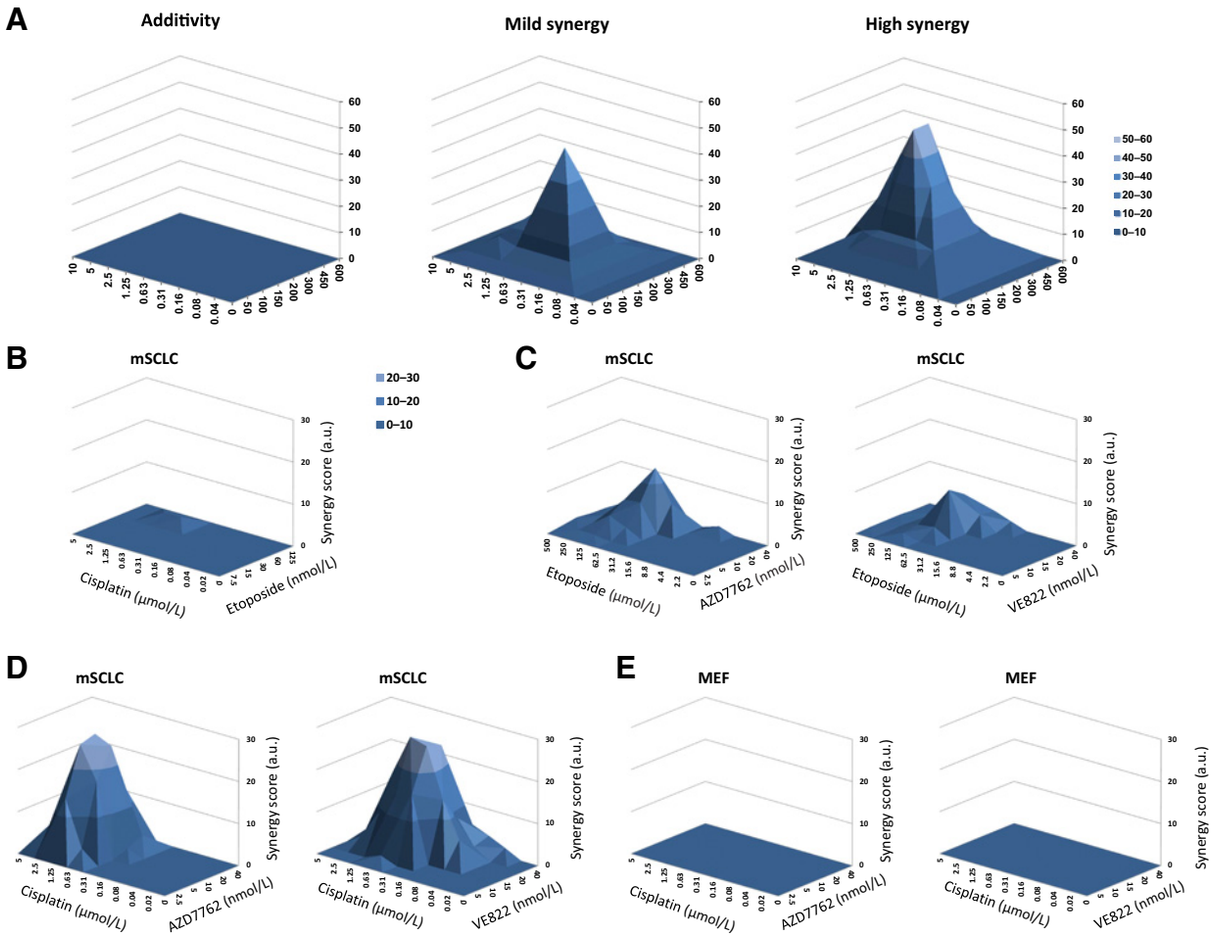

Figure 2.

Replication stress inhibition synergizes with DNA damaging agents in mouse cells. **A,** Graphs showing hypothetical combinations of two drugs in a combination matrix. The different doses of the drugs are indicated on the x- and y-axes. Graphs were produced using MacSynergyII software, which determines the degree of interaction between two drugs. Three potential outcomes are shown, with additive effects represented as a flat plane (left), mild synergism as a peak (middle), and high synergy as a peak with an increase in volume. **B,** Graph showing the synergy score of the combination matrix of cisplatin and etoposide in an mSCLC cell line. **C** and **D,** Synergy scores of the combinations of AZD7762 (CHK1 inhibitor) and VE-822 (ATR inhibitor) with etoposide (**B**) or cisplatin (**C**) in mSCLC. **E,** Synergy scores of the combinations of AZD7762 and VE-822 with cisplatin in primary MEFs. All synergy scores were calculated using the MAC Synergy II algorithm using data from three independent experiments.

bearing DMS-273 xenografts ($P = 2 \times 10^{-3}$), the combination of VE-822 and cisplatin was able to improve the survival of mice bearing tumors originating from either of the cell lines ($P = 1.8 \times 10^{-5}$ and $P = 5 \times 10^{-3}$) (Figure 4B and D).

Discussion

In the current study, we performed a genome-wide CRISPR-Cas9 screen to identify novel druggable targets for SCLC. Because this type of tumor does not harbor obvious druggable targets, it was striking to see that our screen unveiled several cellular processes that are essential for the survival of SCLC cells. First and foremost, we were able to show that the RSS machinery is required for SCLC survival. In addition, the screen also indicated that the mitotic Polo-like kinase signaling might be crucial for normal growth of this type of cancer. This could well be due to the high proliferative capacity of SCLC and the high mitotic index found in these tumors (32). When looking further at the hitlist, there are hints of DNA damage repair being important for SCLC cells. Speculating on the pathways that were found, the nucleotide excision repair pathway could prove to be essential. Furthermore, the high listing of the GO term "hereditary breast cancer signaling" also suggests that homologous recombination could be relevant. The feature of SCLC being sensitive to DNA damage has already been exploited since 1960 where nitrogen mustard was shown to be an effective treatment option for this tumor type (33). Since then, the standard of care has been optimized by combining two other DNA damaging agents, cisplatin and etoposide. The high clinical response rate to this combination indicates that SCLC is highly susceptible to DNA damage. Our screen has also pointed out that the DNA damage response is a synthetic vulnerability for this type of cancer. Unfortunately, the good response to these DNA damaging agents is often followed by a rapid relapse with

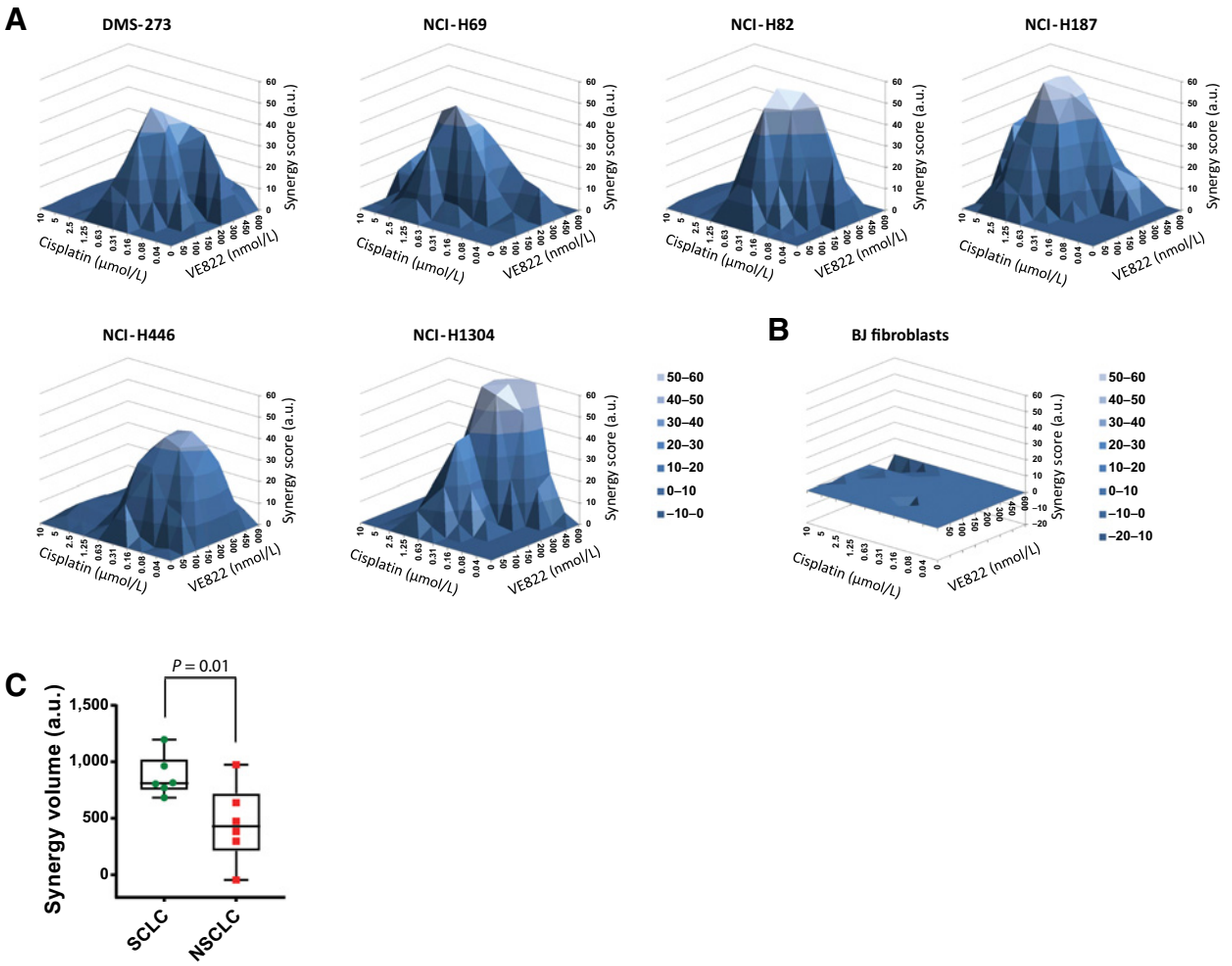

Figure 3.

Replication stress inhibition synergizes with DNA damaging agents in human cells. **A,** Synergy scores of the combination matrix of cisplatin and the ATR inhibitor VE-822 tested in a panel of 6 human SCLC cell lines. **B,** Synergy score of the combination of cisplatin and VE-822 tested on primary human fibroblasts. All synergy scores were calculated using the MAC Synergy II algorithm using data from three independent experiments. **C,** Comparison of the synergy volumes of a panel of 6 SCLC and 6 NSCLC cell lines. Synergy volumes were obtained from the experiments represented in Fig. 3A and Supplementary Fig. S2C. Statistical significance was calculated using a Student *t* test.

resistant disease and thereby warrants the development of novel treatment strategies.

As the RSS genes were highly abundant in the dropouts from the screen (Supplementary Table S5), we followed up on these results by testing the efficacy of ATR and CHK1 inhibitors. These kinases are the sensor kinase for replication stress and its downstream target, respectively (34). Our results show that in SCLC cells, high levels of *MYC* or *MYCL1* expression are associated with sensitivity to CHK1 inhibitors. This observation was extended by showing that SCLC cells are significantly more sensitive to ATR or CHK1 inhibition than nontransformed cells. Together, these results suggest that the sensitivity to ATR or CHK1 inhibition might depend on the presence of high levels of *MYC*-family members. Notably, the presence of a powerful oncogene such as RAS or MYC has previously been shown to lead to replication stress and therefore induces sensitivity to these types of inhibitors (35, 36). Because more than half of SCLC cases harbor amplification and/or overexpression of *MYC*-family members, a large opportunity arises to stratify patient groups for treatment with replication stress inhibitors on the basis of *MYC* and *MYCL1* expression (30, 31). Hereby our findings show a high relevance for clinical application of these inhibitors for SCLC treatment.

Given that etoposide, cisplatin, and ATR/CHK1 inhibitors all act in the S-phase of the cell cycle, the interaction between these different compounds was determined. Whereas the combination of cisplatin and etoposide only showed additive effects *in vitro*, there was a clear synergy between ATR/CHK1 inhibition combined with either cisplatin or etoposide. The synergy between cisplatin and ATR inhibition was extremely high in SCLC cells, but, remarkably, it was completely absent in nontransformed cells. This suggests that these synergistic effects are also likely to depend on high levels of *MYC* expression. In addition, the combination of cisplatin and VE-822 was significantly more

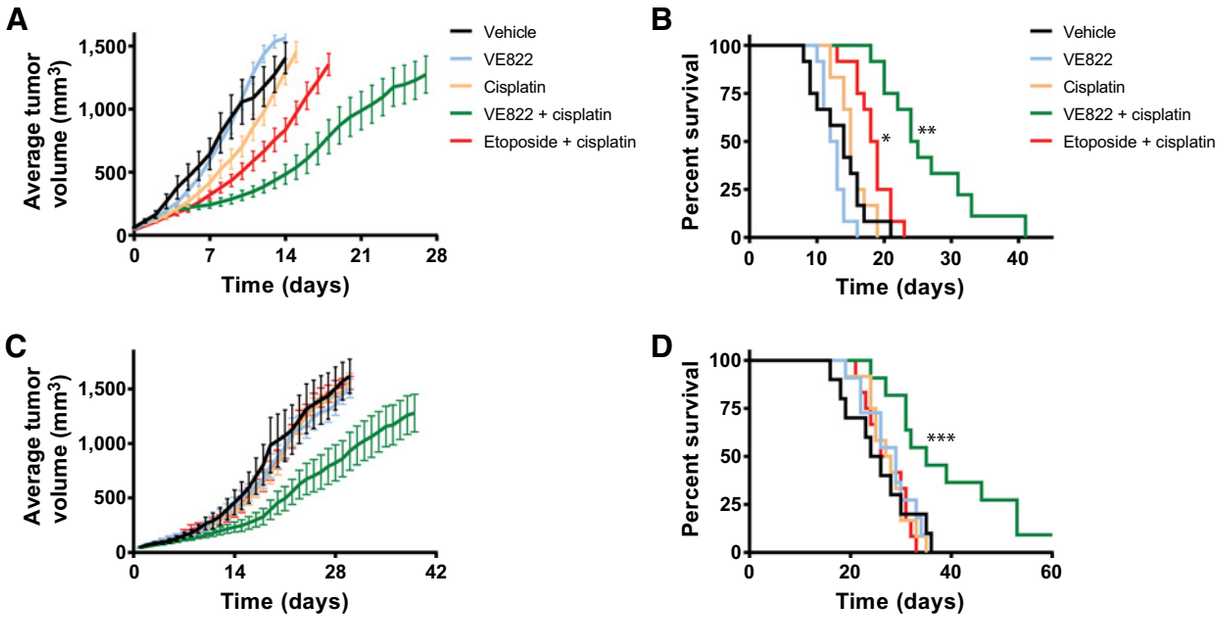

Figure 4.

ATR inhibition in combination with cisplatin *in vivo*. **A,** Graph representing the average tumor volumes ± standard error of the mean (SEM) of xenografted DMS-273 cells. **B,** Survival curve of mice bearing DMS-273 xenografts treated with the indicated compounds. Endpoint of this analysis is the time until the xenografts reach a tumor volume of 1500 mm^3. Average survival: vehicle 13.4 ± 3.9; VE-822 12.5 ± 1.7; cisplatin 15.3 ± 2.3; cisplatin + etoposide 18.3 ± 2.7 and cisplatin + VE-822 26 ± 6.5 days. *, $P = 0.002$; **, $P = 1.8 \times 10^{-5}$. **C,** Average tumor volumes ± SEM of xenografted NCI-H187 cells. **D,** Survival curve of mice bearing NCI-H187 xenografts treated with the indicated compounds. Endpoint of this analysis is the time until the xenografts reach a tumor volume of 1500 mm^3. For all groups, 12 mice were used. Average survival: vehicle 25.5 ± 6.9; VE-822 27.8 ± 5.4; cisplatin 27.4 ± 4.4; cisplatin + etoposide 27.1 ± 4.9 and cisplatin + VE-822 39.2 ± 12 days. ***, $P = 0.005$.

effective in a panel of human SCLC lines compared with human NSCLC, and thus this combination shows high potency against this recalcitrant cancer type *in vitro*. It is interesting to note that the observed synergy is present over a wide range of concentrations, indicating that there is a large window of opportunity to apply these drugs. The cisplatin and VE-822 combination also showed high synergy in an *in vivo* setting with significantly more antitumor activity than the current standard of care. All in all, these results show that the combination of cisplatin and ATR inhibition holds high promise and urges tests of this combination for treatment of SCLC in clinical trials.

A few recent studies have established the efficacy of ATR and CHK1 inhibition in SCLC and even show that its effectiveness in combination with cisplatin against cisplatin-resistant cells (37–39). Here, it was shown that CHK1 is overexpressed in SCLC at both the mRNA and protein levels, leading to an increased sensitivity to CHK1 inhibitors (37, 38). Both studies show that single CHK1 inhibitor treatment *in vivo* has profound effects on tumor growth (37, 38), which is in sharp contrast with our study, where treatment with VE-822 alone does not show any significant effect. This could stem from the choice of xenografted cell lines, as a large variability in the response to the inhibitors can already be observed *in vitro*.

Our data add to the current literature by unveiling that SCLC is critically depending on a functional replication stress machinery, most likely due to high expression of *MYC*-family members, a feature that was also hinted at by Sen and colleagues (38). Whereas high levels of the *MYC* oncogene induce an overall growth advantage, it also generates a massive burden in the form of elevated levels of replication stress. Even though cells normally do not depend on the RSS machinery for their survival, these oncogene-expressing cells do, thereby making it a classic example of a synthetic vulnerability. Especially in the case of SCLC, where very few actionable targets are available, targeting such vulnerabilities becomes an attractive therapeutic approach, even more so because they spare nontransformed cells. A recent clinical phase I study on the tolerability of the ATR inhibitor M6620 in combination with topotecan showed preliminary data that combining inhibition of the RSS machinery with chemotherapy shows clinical benefit especially in SCLC patients (40). In this study 3 out of 5 SCLC patients, who all had platinum-refractory disease, showed a partial response or prolonged stable disease. Therefore, in light of this recent clinical trial, our preclinical data on the targeting of this synthetic vulnerability in SCLC together with cisplatin treatment warrant further clinical validation of this combination.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R. Nagel, A.T. Avelar, H. de Vries, A. Berns
Development of methodology: A.T. Avelar
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R. Nagel, A.T. Avelar, N. Proost, M. van de Ven, J. van der Vliet, M Cozijnsen
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Nagel, N. Aben, H. de Vries, L.F.A. Wessels

Writing, review, and/or revision of the manuscript: R. Nagel, N. Aben, L.F.A. Wessels, A. Berns
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N. Proost, M. van de Ven
Study supervision: A. Berns

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