Combination of PARP and WEE1 inhibitors *in vitro*: Potential for use in the treatment of SHH medulloblastoma

MONIKA LUKOSEVICIUTE, AIKATERINI THEODOSOPOULOU, STEFAN HOLZHAUSER, TINA DALIANIS and OURANIA N. KOSTOPOULOU

Department of Oncology-Pathology, Karolinska Institutet, 171 64 Stockholm, Sweden

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Abstract. Medulloblastoma (MB), grouped as either WNT-activated, Sonic hedgehog (SHH)-activated, or non-WNT/non-SHH group 3, accounts for almost 20% of all childhood brain cancers. In spite of current intensive treatments, not all patients are cured and survivors suffer from severe side-effects. The present study therefore examined the effects of the poly-ADP-ribose polymerase (PARP) and WEE1-like protein kinase (WEE1) inhibitors, BMN673 and MK-1775, respectively, alone or in combination on four MB cell lines. More specifically, the MB cell lines, DAOY, UW228-3, MED8A and D425, were tested for their sensitivity to BMN673 and MK-1775 alone or in combination, using cell viability, cell confluency and cytotoxicity assays. The effects on the cell cycle phases were also examined using FACS analysis. Monotherapy with BMN673 and MK-1775 exerted dose-dependent inhibitory effects on the viability of almost all MB cell lines. Notably, when BMN673 and MK-1775 were used in combination, synergistic effects were noted in the SHH group cell lines (DAOY and UW228-3), but not in the already WEE1-sensitive group 3 (MED8A and D425) lines. Moreover, the combination treatment decreased the percentage of cells in the G1 phase and induced the novel distribution of both DAOY and UW228-3 cells in the S and G2/M phases, with the UW228-3 cells exhibiting a greater delay. To conclude, MK-1775 was efficient in all and BMN673 in most cell lines, and their combined use exerted synergistic effects on the SHH, but not the group 3 cell lines. These data suggest that MK-1775 alone may be of interest for all MB cell lines, and that the combination of PARP/WEE1 inhibitors may provide possible therapeutic opportunities for the therapy of SHH MBs. Their use warrants further investigations in the future.

Correspondence to: Dr Ourania N. Kostopoulou, Department of Oncology-Pathology, Karolinska Institutet, Bioclinicum J6:20, 171 64 Stockholm, Sweden
E-mail: ourania.kostopoulou@ki.se

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Introduction

Medulloblastoma (MB) is a highly malignant and invasive childhood brain tumor most predominantly located in the cerebellum (1-4). Currently, the majority of patients with MB undergo surgical resection and receive multi-agent chemotherapy, while children >3 years of age are also treated with craniospinal radiation therapy (5,6). Although current treatment strategies have increased the average survival of rate of patients with MB up to 70%, the major disadvantages of such therapies include the development of drug resistance, metastasis, disease recurrence which is universally fatal, and long-term toxicities (7-9). There is therefore a need for the development of novel personalized targeted therapies focusing on molecular alterations and individual tumor molecular profiles.

According to the renewed 2021 World Health Organization (WHO) Classification of Tumor of the Central Nervous System, MB is classified into four genetically-defined subgroups, including WNT-activated (best prognosis), Sonic hedgehog (SHH)-activated TP53 wild-type, SHH-activated TP53-mutant, and non-WNT/non-SHH (group 3, worst prognosis; and group 4, intermediate outcomes) (10-17).

The understanding of specific tumorigenic mutations, molecular drivers and deregulated signaling pathways in molecular subgroups accelerates the discovery of potential therapeutic targets. Potential targets are poly(ADP-ribose) (PAR) polymerase (PARP)1 and PARP2, which are known as the key proteins coordinating the DNA damage response, specifically the damage detection and repair of both single- and double-strand breaks (18-21). In particular, PARP1 and PARP2 (PARP1/2) are involved in the synthesis of PAR chains, which in turn recruit DNA repair proteins. PARP inhibitors have therefore been actively used in the treatment of cancers harboring defects in homologous recombination, such as in tumors with *BRCA1* and *BRCA2* mutations, thereby leading to synthetic lethality (22,23). Among the PARP inhibitors, BMN673 (talazoparib) is the most potent selective PARP1/2 inhibitor (24,25). BMN673 is currently approved by the US Food and Drug Administration (FDA) for the treatment of *BRCA1* or *BRCA2* mutated, negative HER2 locally advanced or metastatic breast cancer (26). In other clinical studies, an ongoing phase II trial is testing the effects of BMN673 in patients with recurrent high-grade glioma (NCT04740190).

Although BMN673 has not extensively been tested in MB, previous studies have demonstrated that other PARP inhibitors, such as olaparib, rucaparib and veliparib are potential chemo- and radiosensitizing agents in MB cells and xenograft models (27-29).

Another promising molecular target is the WEE1-like protein kinase (WEE1), which is involved in the regulation of the S phase and G2/M checkpoint of the cell cycle (30-32). In response to DNA damage, WEE1 mediates the inhibition of the phosphorylation of cyclin-dependent kinase (CDK) 1 and CDK2, resulting in cell cycle arrest and possible DNA repair (33,34). Multiple studies have reported increased expression levels of WEE1 in various types of cancer, including pediatric high-grade gliomas, glioblastoma, ovarian cancer, melanoma, breast cancer and MB (33,35-38). The inhibition of WEE1 can impair the G2/M checkpoint, allowing cancer cells with DNA damage to divide, eventually leading to mitotic catastrophe (39). Currently, MK-1775 is the first selective small-molecule inhibitor of WEE1, which has exhibited promising antitumor efficacy, when combined with chemotherapeutics agents, against pancreatic, breast, colon and ovarian cancer (40-43). In addition, it has been reported that MK-1775 is highly effective in tumor cells harboring *p53* mutations (44,45). Specifically, in MB, previous research has demonstrated that MK-1775 single treatment can inhibit MB tumor growth *in vivo* (46).

The crucial roles of PARP and WEE1 in the DNA damage response render them potential therapeutic targets against MB tumors. The present study focused on targeting proteins that are critical for DNA damage repair, potentially making this treatment strategy applicable to different molecular subgroups. More specifically, the present study examined the effects of the FDA-approved PARP inhibitor, BMN673, and the not yet FDA-approved WEE1 inhibitor, MK-1775, as single agents and in combination on four MB cell lines DAOY, UW228-3, MED8A and D425.

Materials and methods

Tumor cell lines and cell seeding. The MB cell lines DAOY, UW228-3 (both group SHH) and D425 and MED8A (both group 3), were obtained from Professor Per Kogner, Karolinska Institutet and cultured in minimum essential medium (MEM), Dulbecco's modified Eagle's medium (DMEM):nutrient mixture F-12 (DMEM/F-12) and DMEM with GlutaMAX (both Group 3 cell lines), respectively, with the addition of 10% fetal bovine serum (FBS) (all from Gibco; Thermo Fisher Scientific, Inc.), together with 1% L-glutamine, 100 U/ml of penicillin as well as 100 μg/ml streptomycin (Thermo Fisher Scientific, Inc.). The DAOY, UW228-3 and D425 cells are p53 mutated and D425 and MED8A have a MYC amplification (47) (https://www.cellosaurus.org/CVCL_1275; https://www.cellosaurus.org/CVCL_M137). For the viability and proliferation/cytotoxicity assays, $2.5x10^3$ cells/well were plated for the DAOY cells, $5x10^3$ cells/well were plated for the UW228-3 cells, and 10^4 cells/well were plated for the D425 and MED8A cells (group 3 were grown in suspension) in 90 and 200 μl medium in 96-well plates, respectively. For western blot (WB) and FACS analyses, $5x10^5$ cells/Ti25 flask in 5 ml medium were plated for both the DAOY and UW228-3 MB cell lines.

Inhibitors. The PARP inhibitor, BMN673 (talazoparib), the WEE1 inhibitor, MK-1775 (AZD-1775, adavosertib), the phosphoinositide 3-kinase (PI3K) inhibitor, BYL719 (alpelisib), and the CDK4/6 inhibitor, PD-0332991 (palbociclib), in DMSO stock solutions (Selleck Chemicals GmbH), were used in various dilutions in PBS [further details have been previously described (47,48)].

WST-1 viability assay. Following the addition of (0.1, 0.5, 1 and 10 MK-1775 and BMN673 and their combinations), for 24, 48 and 72 h, the viability, i.e., the estimation of remaining live healthy cells in response to therapy of the cell population was estimated using WST-1 viability assay (Roche Diagnostics GmbH) as previously described in more detail (47,48).

Cell confluency, cytotoxicity and apoptosis assays. The IncuCyte S3 Live Cell Analysis System was used to examine the cell confluency, as a measure of proliferation following treatment and cytotoxicity, as a measure of cell damage following treatment of the DAOY and UW228-3 cells, both grown as monolayers (47,48). More specifically, at 24 h after seeding, the medium was changed to a new medium containing the Incucyte™ Cytotox Red Reagent (Essen Bioscience), that enters the damaged plasma membrane and binds to DNA in the nuclei, the treatments were then added and the plates were incubated at 37˚C in the machine for 72 h. Images were collected every 2 h to follow cell confluence/proliferation with IncuCyte S3 Live Cell Analysis System (Satorius). Cytotoxicity was quantified by counting the red nuclei. Apoptosis was also assayed in the DAOY and UW228-3 cells using the IncuCyte S3 Live Analysis System by the addition of the IncuCyte Caspase-3/7 Green Apoptosis reagent, that enters live cells, as previously described in further detail (47,48).

FACS analysis. For cell cycle analysis, the cells were collected following 48 h of treatment and fixed with 70% ethanol. A total of $5x10^5$ cells were counted and stained with FxCycleR/RNAse solution (Invitrogen™, ThermoFisher Scientific, Inc.). All samples were analyzed with the FACS NovoCyte 3000, while the analysis of the data was conducted using FlowJo_v10.8.1 software (BD Biosciences).

Statistical analysis. All the results were subjected to statistical analysis. To estimate the efficacy of the single or combination treatments compared to the negative control, a multiple t-test accompanied by the correction for multiple comparisons of the means using the Holm Sidak method were used as previously described (49). To investigate the efficacy of the drug combinations the Synergy FinderPlus computational tool (https://synergyfinderplus.org/#!/) with the highest single agent (HSA) was used. HSA values >10 indicated synergistic effects of the drugs, HSA values from -10 to 10 indicated additive effects, and HSA values <-10 indicated antagonism (50).

Results

Effects of single and combination drug treatment with WEE1 and PARP inhibitors on MB cell lines measured using WST-1 assays. All MB cell lines (DAOY, UW228-3, D425 and MED8A) exhibited a concentration-dependent inhibition of

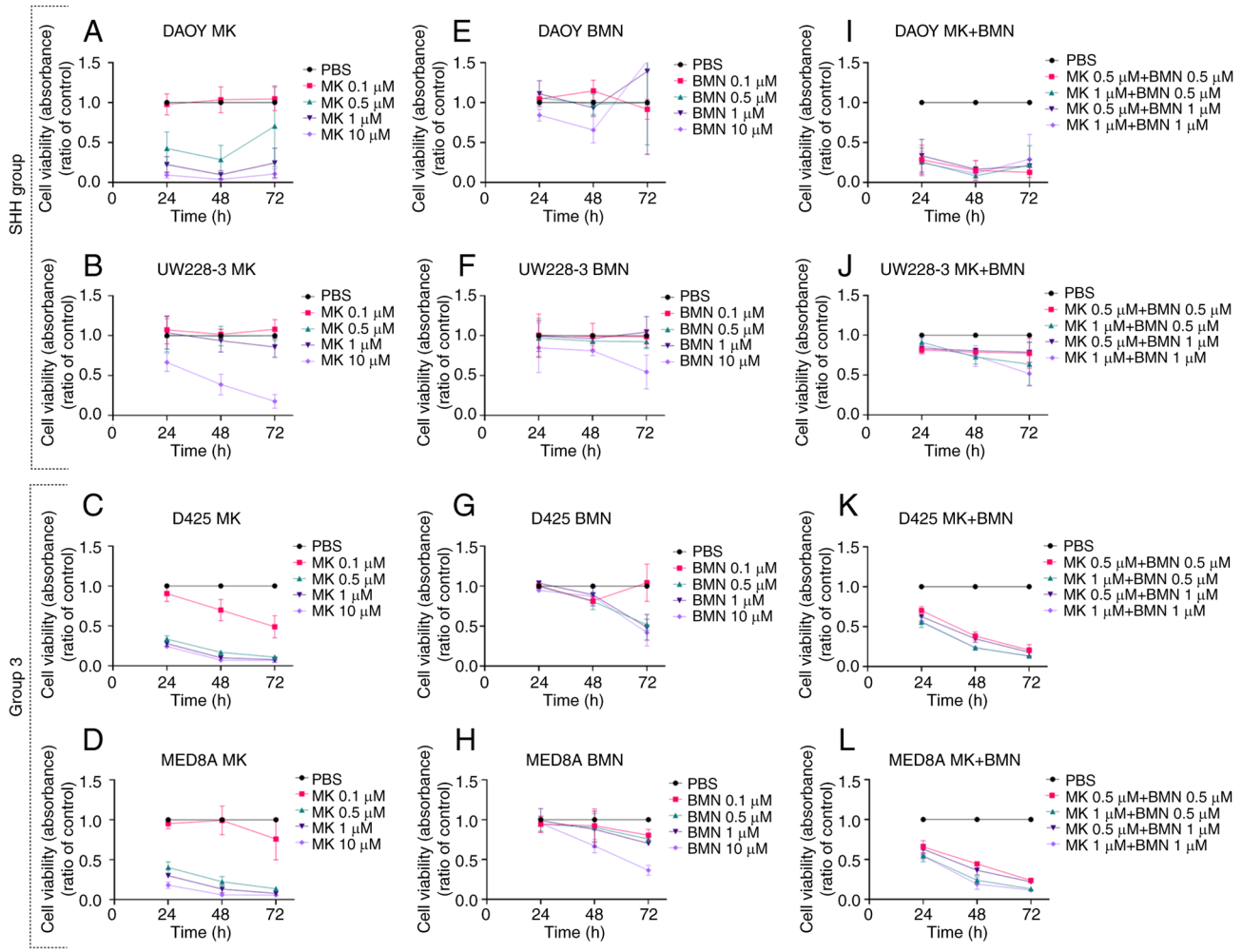

Figure 1. Effects of WEE1 (MK-1775) and PARP (BMN673) single and combination treatments on the viability of DAOY, UW228-3 (SHH group), D425 and MED8A (group 3) medulloblastoma cell lines. The absorbance was measured 24, 48 and 72 h following the addition of (A-D) MK-1775, (E-H) BMN673 or (I-L) their combination. The graphs represent at least three experimental runs per cell line. MK, MK-1775; BMN, BMN673.

viability at 24-72 h following treatment (determined using WST-1 assays) with the WEE1 inhibitor, MK-1775 (0.1-10 μM), and likewise, with the exception of DAOY cells, with the PARP inhibitor, BMN673 (0.1-10 μM).

MK-1775. All MB cell lines exhibited a >50% significant decrease in viability in comparison to PBS control with the highest concentration (10 μM) of the WEE1 inhibitor, MK-1775, at almost all time points following treatment (for all at least P<0.005) (Fig. 1A-D). In addition, all MB cell lines, apart from the UW228-3 cells, exhibited a >50% decrease in viability compared to the PBS control following treatment with 0.5 and 1 μM MK-1775 at most time points (for all those at least P<0.05) (Fig. 1A-D).

BMN673. Only the highest concentration (10 μM) of the PARP inhibitor, BMN673, led to a >50% decrease in viability in comparison to the PBS control at 72 h following treatment in all MB cell lines, apart from the DAOY cells (for all those at least P<0.001) (Fig. 1E-H).

MK-1775 and BMN673 in combination. All MB cell lines, apart from the UW228-3 cells, exhibited a >50% decrease in viability compared to the PBS control at both 48 and 72 h following treatment with all drug combinations, while the UW228-3 cells only exhibited a significant decrease with the highest combination concentrations at 72 h following treatment (for all at least P<0.05) (Fig. 1I-L).

To summarize, all four MB cell lines exhibited concentration-dependent responses to both inhibitors with the exception of the DAOY cells to BMN673, and with MK-1775 being more efficient than BMN673 at the drug concentrations used. Moreover, upon combining the inhibitors, the UW228-3 cells were generally more resistant as compared to the other three MB cell lines.

Synergistic effects of combined drug treatment with WEE1 and PARP inhibitors on MB cell lines. Based on the cell viability data described above, the synergy between WEE1 and PARP inhibitors was examined using the Synergy FinderPlus program. To examine the synergy, the synergy scores for the four MB cell lines treated with a range of combinations of WEE1 and PARP inhibitors were calculated (Fig. 2). HSA values >10 indicated synergistic effects of the drugs, HSA values from -10 to 10 indicated additive effects, and HSA values <-10 indicated antagonism (50).

The calculations obtained revealed that synergy was most clearly found when combining MK-1775 and BMN673 in the DAOY and UW228-3 cells, while the same drug combinations exerted mainly antagonistic effects in the D425 and MED8A

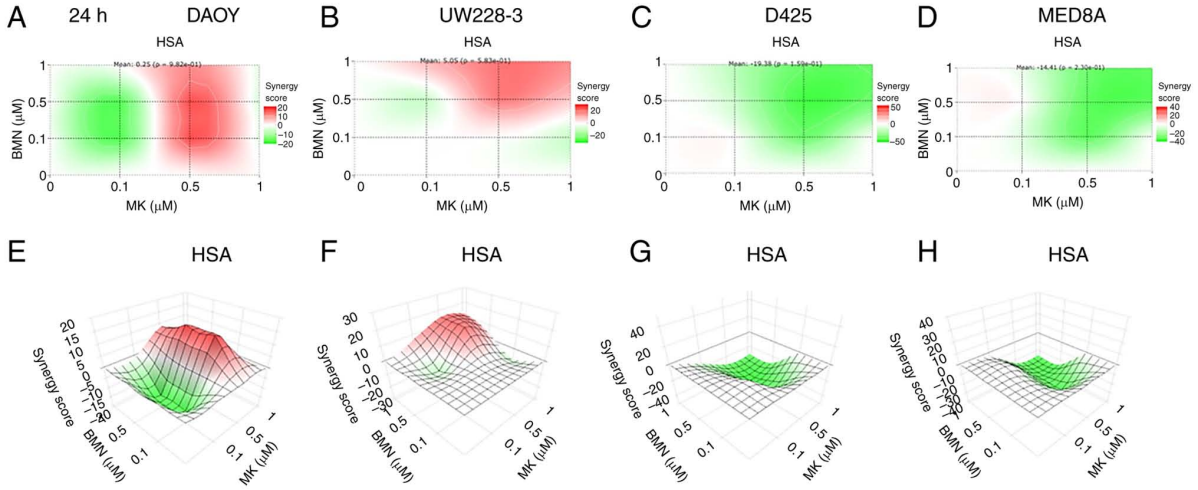

Figure 2. Representation of (A-D) 2D and (E-H) 3D surface plots illustrating the optimal concentrations of MK-1775 and BMN673 in medulloblastoma cell lines. The synergy score was calculated using the Synergy Finder plus program. HSA values >10 indicated synergistic effects of the drugs, HSA values from -10 to 10 indicated additive effects, and HSA values <-10 indicated antagonism. MK, MK-1775; BMN, BMN673; HSA, highest single agent.

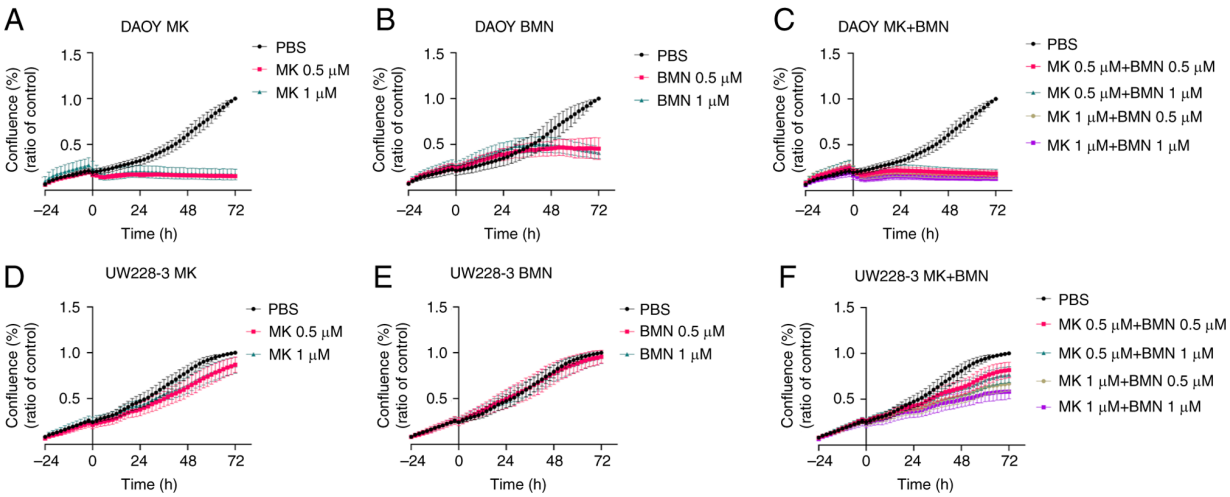

Figure 3. Effects of with WEE1 (MK-1775) and PARP (BMN673) inhibitors alone and in combination on the confluency of DAOY and UW228-3 medulloblastoma cell lines. The cells were followed for up to 72 h after treatment. Single treatments are presented for (A and D) MK-1775 and for (B and E) BMN673. (C and F) Combined treatments are presented. MK, MK-1775; BMN, BMN673.

cells (Fig. 2). Since synergy was optimal in the UW228-3 and DAOY cells, these two cell lines were selected for use in further experiments.

Other combinations. MK-1775 was also combined with BYL719 and PD-0332991, two other regulators of the cell cycle, in order to examine the joint effects of MK-1775 with BYL719 or PD-0332991 on DAOY and UW228-3 cells. However, at the concentrations used (5 and 10 μM of BYL719, and 5 and 10 μM of PD-0332991), no major enhancements were observed as compared to the effect of MK-1775 alone, with possibly one exception of a slight enhancement using MK-1775 and PD-0332991 in combination on the UW228-3 cells (data not shown). These data were therefore not pursued further herein.

Cell confluency and cytotoxicity following treatment of the DAOY and UW228-3 cells with WEE1 and PARP inhibitors either alone or in combination. The effects of treatment with the MK-1775 and BMN673 inhibitors either alone or in combination on the DAOY and UW228-3 cells were further analyzed up to 72 h following treatment using cell confluency and cytotoxicity assays utilizing the IncuCyte S3 Live-Cell Analysis System (Figs. 3 and 4).

Cell confluency

MK-1775. For the DAOY cells, both the MK-1775 concentrations (0.5 and 1 μM) used induced an almost complete reduction in cell confluency as compared to the PBS control, while for the UW228-3 cells, only a marginal reduction in cell confluency was noted (Fig. 3A and D).

BMN673. For the DAOY cells, both concentrations (0.5 and 1 μM) of BMN673 used resulted in a reduced cell confluency compared to the PBS control, while for the UW228-3 cells, no effect on cell confluency was observed (Fig. 3B and E).

MK-1775 and BMN673. For the DAOY cells, all drug combinations resulted in an almost complete reduction of cell confluency compared to the PBS control, while for the

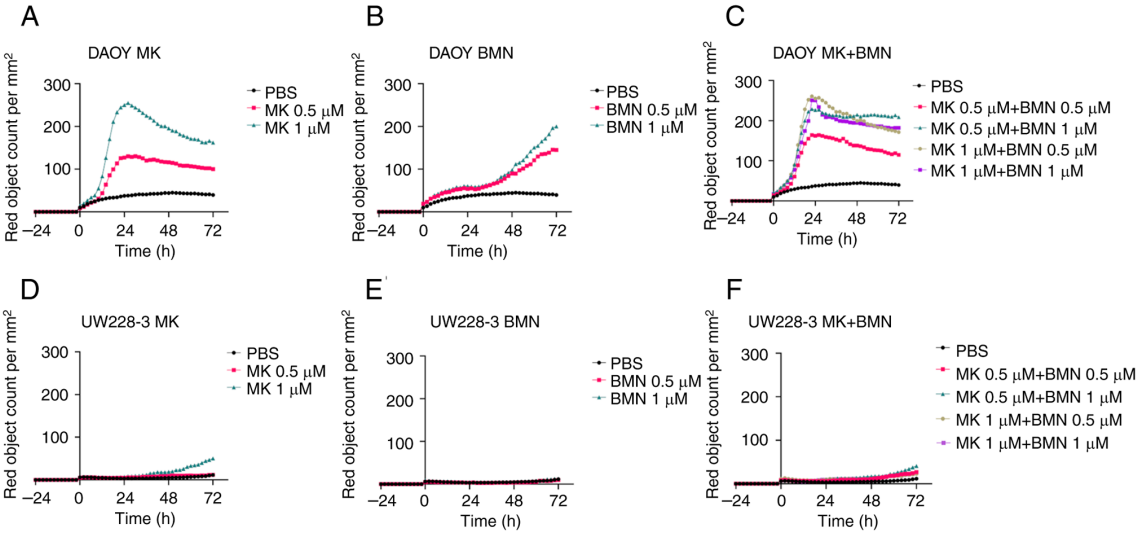

Figure 4. Cytotoxic effects of WEE1 (MK-1775) and PARP (BMN673) inhibitors alone and in combination on DAOY and UW228-3 medulloblastoma cell lines. The cytotoxic effects of the inhibitors on the DAOY and UW228-3 cells were measured for up to 72 h after treatment. Single treatments are presented for (A and D) MK-1775 and for (B and E) BMN673. (C and F) Combined treatments are presented. The graphs represent one experimental run per cell line. MK, MK-1775; BMN, BMN673.

UW228-3 cells, the effects were concentration-dependent and less pronounced (Fig. 3C and F).

To conclude, MK-1775 alone considerably reduced the confluency of the DAOY cells and this was also the case, to a certain extent, for cell confluency upon treatment with BMN673; an enhanced was not detected for the DAOY cells following combination treatment. By contrast, while almost no effect on UW228-3 cell confluency was observed upon treatment with the inhibitors alone, when used in combination, the inhibitors led to a concentration-dependent inhibition of cell confluency.

Cytotoxicity

MK-1775. Both the MK-1775 concentrations (0.5 and 1 μM) exerted marked cytotoxic effects on the DAOY cells, whereas no marked cytotoxic effects were observed on the UW228-3 cells (Fig. 4A and D).

BMN673. Both the BMN673 concentrations (0.5 and 1 μM) exerted some cytotoxic effects on the DAOY cells, whereas no cytotoxic effects were observed on the UW228-3 cells (Fig. 4B and E).

MK-1775 and BMN673. The combined use of MK-1775 with BMN673 did not exert any exerted enhanced cytotoxic effects on either the DAOY or UW228-3 cells as compared to using the most efficient single inhibitor, MK-1775 (Fig. 4C and F).

To investigate this further, and to determine whether part of the cytotoxic response was due to apoptosis, an apoptosis assay was also performed. However, no major effects on apoptosis were observed with any of the single or combined drug administrations used above (Fig. S1).

To conclude, although treatment with MK-1775, but also BMN673 alone exerted cytotoxic effects on the DAOY cells, their combined use did not exert any enhanced effects. In the UW228-3 cells, none of the single or combined treatments exerted pronounced cytotoxic effects.

Effects of PARP and WEE1 inhibitors alone or in combination on the cell cycle progression of MB cells. The effects on cell cycle progression were examined using a FACS NovoCyte 3000 machine and FlowJo_v10.8.1 software. More specifically, the effects of WEE1 and PARP inhibitors alone or in combination (MK-1775 and BMN673) were examined on the SHH cell lines, DAOY and UW228-3 (Figs. 5 and S2).

MK-1775. Both single MK-1775 concentrations (0.5 and 1 μM) induced an increase in the proportion of cells in the S and G2 phases (compared to the PBS control), with a higher percentage of cells arrested in the G2 phase using the 1 μM concentration in both cell lines, although with a higher amount of UW228-3 cells remaining in the G1 phase in comparison to the DAOY cells (Fig. 5).

BMN673. Both single BMN673 concentrations (0.5 and 1 μM) induced an increase in the proportion of cells in the S phase and to a lower extent in the G2 phase (compared to the PBS control) in both cell lines, although with a higher number of UW228-3 cells remaining in the G1 phase in comparison to the DAOY cells (Fig. 5).

MK-1775 and BMN673. All MK-1775 and BMN673 combinations, irrespective of the concentrations used, led to a decrease in the percentage of cells in the G1 phase, with a clear shift of the cells from the S to the G2 and >G2 phases in both the DAOY and UW228-3 (Fig. 5).

To conclude, the single MK-1775 and BMN673 administrations increased the proportion of cells in the S and G2 phases in both cell lines, and their combination exerted additive effects on both cell lines; however, the shifts in cell cycle progression were generally delayed in the UW228-3 cells when compared to the DAOY cells.

Discussion

In the present study, WEE1 and PARP inhibitors (MK-1775 and BMN673, respectively) were evaluated alone or in combination in four MB cell lines, namely DAOY, UW228-3 (both group SHH), and D425 and MED8A (both group 3). All MB cell lines exhibited, at the concentrations used, concentration-dependent responses, with a decrease in viability upon single MK-1775

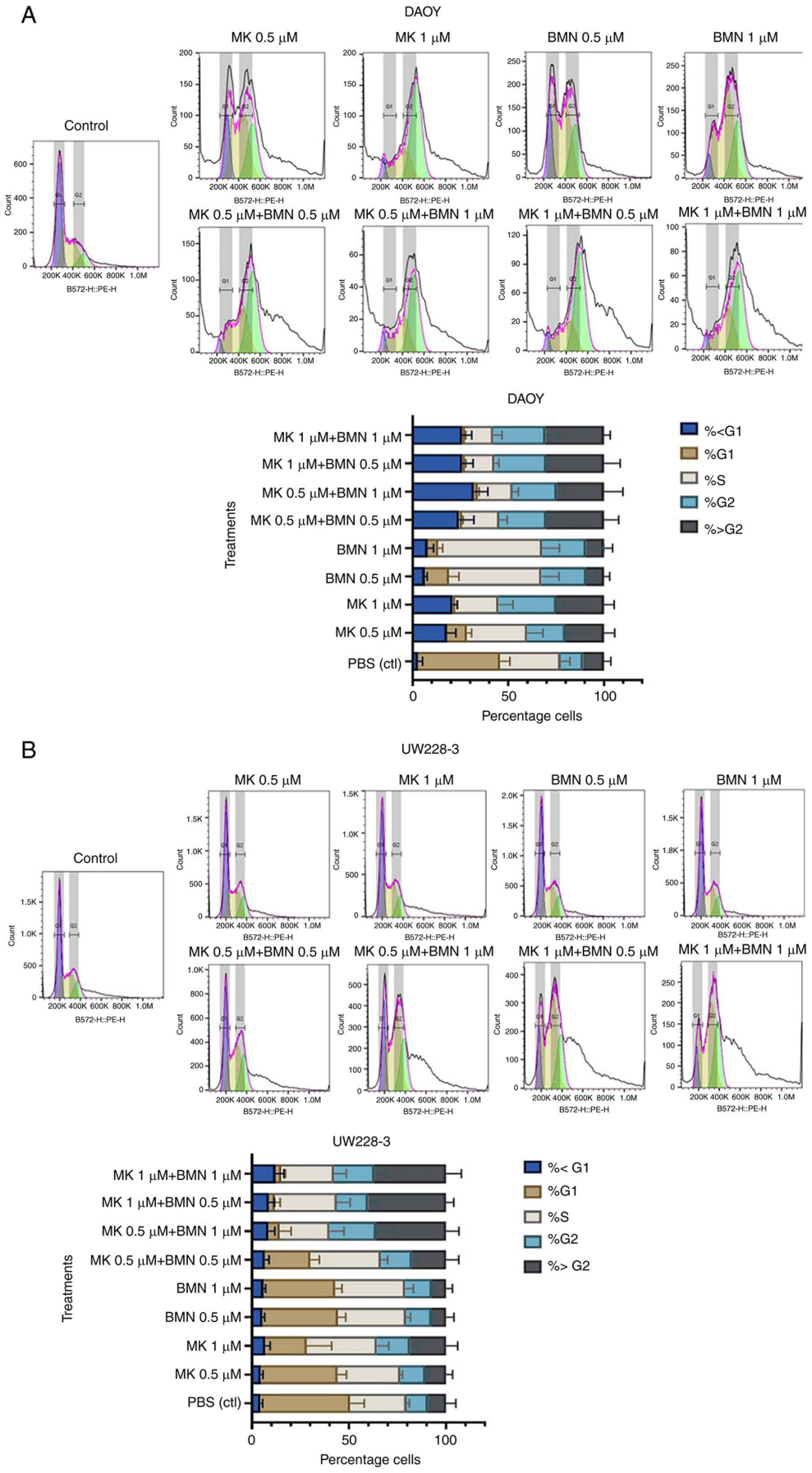

Figure 5. Cell cycle profiles and analysis of each cell cycle phase of DAOY and UW228-3 MB cell lines following treatment with WEE1 (MK-1775) and PARP (BMN673) inhibitors alone or in combination for 48 h. Representative cell cycle profiles of (A) DAOY and (B) UW228-3 MB cell lines are shown on the upper panel and the percentages of cell distribution in the different cell cycle phases are presented on the bottom panel. The percentages represent three experimental runs per cell line. Ctl, control; BMN, BMN673; MK, MK-1775, MB, medulloblastoma.

treatment and with the exception of the DAOY cells, which to certain to a certain extent, also exhibited a response to BMN673 treatment. Furthermore, upon combining the two drugs, synergy was noted in the DAOY and UW228-3 cells (both group SHH), but not in the group 3 cell lines; thus, only the former two cell lines were examined in further detail for the effects of single and combined inhibitor treatments on cell confluency, cytotoxicity and the cell cycle.

Presently, the authors have no explanation as to why synergy with regard to viability upon combination treatments with MK-1775 and BMN673 was not obtained in group 3 MB cell lines. One could have argued that this could have been due to the fact that the two SHH cell lines, DAOY and UW228-3, have *p53* mutations; however, this was also the case for the D425 cells; thus, obviously, this is an issue that warrants further investigation in future studies.

Notably, at the concentrations used, MK-1775 alone was superior to BMN673 upon single treatments as regards its effects on viability in all cell lines. A similar outcome was also observed in cell confluency and cytotoxicity, where the DAOY cells were more sensitive than the UW228-3 cells, which in turn exhibited marginal effects with regard to both cell confluency and cytotoxicity. The data obtained herein for BMN673 are thereby in line with previous data on BMN673 in other childhood cancers; a previous study demonstrated limited clinical activity and suggested that single treatment with BMN673 possibly would be more efficient in patients whose tumors had defects in homologous recombination repair (51).

When MK-1775 and BMN673 were used in combination, synergy was disclosed, particularly as mentioned above, in viability, in which a reduction was observed in both the DAOY and UW228-3 cells, while the synergistic effects on cell confluency mainly applied to the UW228-3 cells, where the single drugs had limited effects. On the other hand, a synergistic effect on cytotoxicity was not detected in any cell line, although the DAOY cells were generally more sensitive than the UW228-3 cells, corresponding to similar findings from previous research by the authors using other inhibitors (47).

The concentration-dependent effects which were observed on the MB cell lines with single MK-1775 treatments were expected, since this has been previously demonstrated with corresponding inhibitors on MB and other tumor cell lines, such as head and neck cancer (46,52-55).

Previous analysis with other PARP inhibitors, such as olaparib, rucaparib and veliparib has shown that they are potential chemo- and radiosensitizing agents in MBs, even though there are, to the best of our knowledge, no other publications available using BMN673 on MB (27-29). Furthermore, to the best of our knowledge, there are no other studies available examining the MK-1775 and BMN673 combinations in MB cell lines. Nevertheless, it is known that WEE1 inhibition is efficient in cell lines exhibiting *p53* mutations, although the response is not always only related to a *p53* mutation alone (56).

In addition, in the present study, as mentioned above in the two SHH MB cell lines, the effects on cell cycle progression were examined, since both MK-1775 and BMN673 induce G2/M cell cycle arrest (30-32,57). Following single MK-1775 and BMN673 treatments, there was a decrease in the proportion of cells in the G1 phase in both the DAOY and UW228-3 cells, and an increase in the proportion of cells shifting towards the S and G2/M phase, which is in accordance with recent study, particularly for MK-1775, since it exerts cytotoxic effects in both the S and G2/M phase (56).

Combined treatment with MK-1775 and BMN673 exerted synergistic effects on both cell lines and further decreased the proportion of cells in the G1 and S phases, and increased the number of cells in the G2/M phase. Of note, combined treatment in both cell lines resulted in a clear increase in the proportion of cells in the >G2 phase; this is not surprising, as previous studies on MK-1775 have suggested that WEE1 inhibition causes an impairment in cytokinesis, leading to tetraploid cells (55). This needs to be examined further in order to validate the obtained results. When comparing DAOY and UW228-3 with regard to shifting towards the G2/M phase, the responses of the UW228-3 cells were generally more delayed as compared to those of the DAOY cells. The reason for this currently remains unknown, apart from the fact that the UW228-3 cells are generally more resistant than the DAOY cells (47,48).

Notably, since it has been previously demonstrated that targeted therapy may cause problems in G1 control and can lead to cancer cells becoming dependent on the G2 control to repair DNA damage (58), targeting the G2 checkpoint could be proposed as a possible additional anticancer strategy. Based on the aforementioned hypothesis, the present study combined the WEE1 inhibitor, MK-1775, with the FDA-approved PI3K and CDK4/6 inhibitors, BYL719 and PD-0332991; however, at the concentrations used, only slight synergistic effects were observed with MK-1775 and PD-0332991 on the UW228-3 cells (data not shown); thus, this was not pursued further herein.

There were some limitations to the present study, since only a small number of inhibitors and cell lines were used. Nevertheless, of note, the obtained data demonstrate that drug-drug interactions using WEE1 and PARP inhibitors are complex and can result in either synergistic or antagonistic interactions, depending on the MB subgroup profile and the mutation profile of the different cell lines. While broader concentration ranges and modified incubated periods may shed further light on the drug interactions with respect to their antitumor efficacy, the concentrations used herein adhere to commonly used standard conditions, and therefore allow for more direct comparisons (47,55).

Further and more detailed studies are warranted in order to disclose the possible mechanisms underlying the tested drug combinations exerting synergistic or antagonistic effects and to provide a pre-clinical rationale of how to apply the corresponding combinations clinically. Nevertheless, combining the WEE1 inhibitor, MK-1775, and the PARP inhibitor, BMN673, in two SHH MB cell lines, exerted synergistic effects and allowed for the use of lower inhibitor concentrations compared to those of single treatments, and this could possibly reduce some side-effects. Moreover, targeting MB with two different mechanisms may decrease the risk of resistance.

In conclusion, the present study suggests that treatment with WEE1 alone can have effects on SHH and group 3 MB, and combining WEE1 and PARP inhibitors may be of potential interest for the treatment of the SHH MB group.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ML, AT and SH performed the majority of the experiments, interpreted the data, performed the statistical analyses and contributed to the writing of the manuscript. ML contributed together with ONK to the graphs of the manuscript. ML and SH initiated the experiments and the interpretation of the initial experiments, and contributed to the writing of the material and methods section, all under the supervision of ONK. TD and ONK made substantial contributions to the conception and design of the study, as well as to the acquisition of data, and analysis and interpretation of data, and were also involved in the drafting of the manuscript and revising it critically for important intellectual content. TD also provided the resources for the performance of the experiments such as laboratory space and consumables. TD and ONK were responsible for obtaining financial support for conducting the research Project. All authors critically read and approved the manuscript. ML and ONK confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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