

Alternative lengthening of telomere-based immortalization renders H3G34R-mutant diffuse hemispheric glioma hypersensitive to PARP inhibitor combination regimens

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Abstract

Background. Diffuse hemispheric glioma, H3 G34R/V-mutant (DHG-H3G34) is characterized by poor prognosis and lack of effective treatment options. DHG-H3G34R further harbor deactivation of alpha-thalassemia/mental retardation syndrome X-linked protein (ATRX; DHG-H3G34R_ATRX) suggesting a unique interaction of these 2 oncogenic alterations. In this study, we dissect their cell biological interplay, investigate the impact on telomere stabilization, and consequently validate a targeted therapy approach.

Methods. We characterized patient-derived primary pediatric high-grade glioma (pHGG) models for telomere-maintenance mechanisms, DNA damage stress (including protein expression, pH2AX/Rad51 foci, cell-cycle arrest) and their sensitivity towards poly-ADP ribose polymerase inhibitor (PARPi) combinations. Human induced pluripotent stem cells (iPSCs) were used for modeling the disease. The anticancer activity of PARPi combinations in vivo was studied in Chorioallantoic Membrane (CAM) and orthotopic in vivo experiments. Finally, we treated a DHG-H3G34R_ATRX patient with PARPi combination therapy.

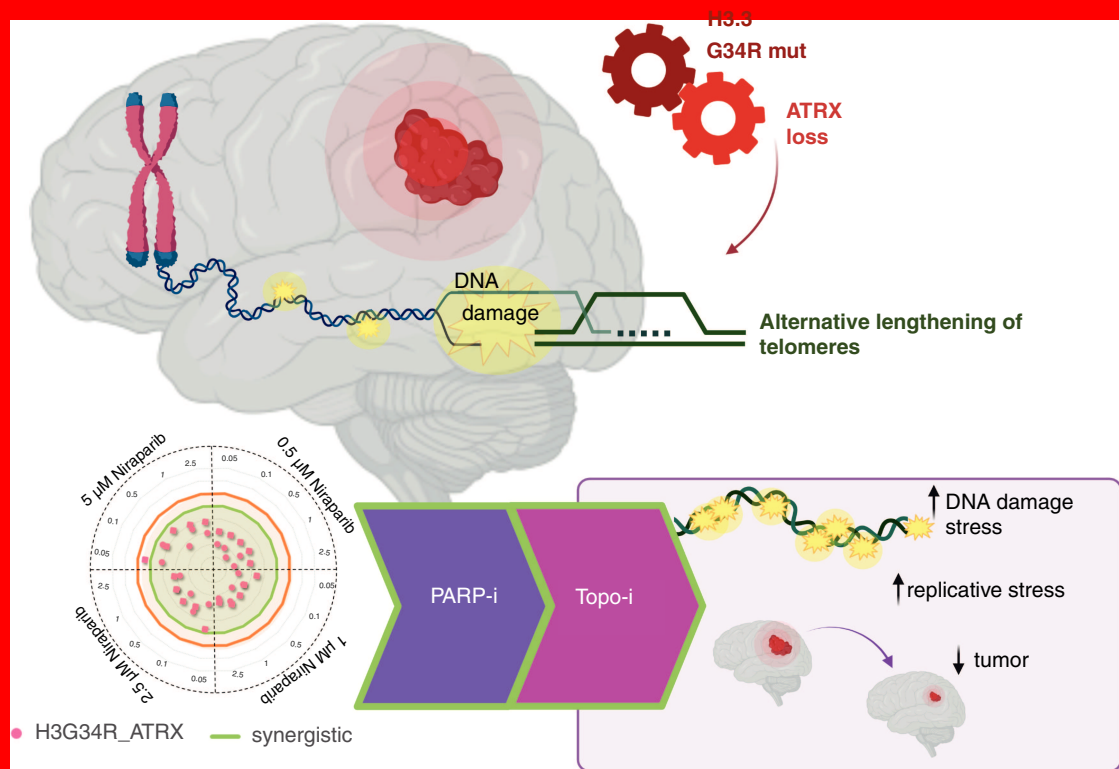
Results. We elaborate that alternative lengthening of telomeres (ALT) is a key characteristic of DHG-H3G34R_ATRX. A dominant cooperative effect between *H3G34R* and *ATRX* loss in ALT activation also became apparent in iPSCs, which endogenously exert telomerase activity. In both, patient-derived DHG-H3G34R_ATRX models and H3G34R+/ATRX- iPSCs, the ALT-phenotype was associated with increased basal DNA damage stress, mediating synergistic susceptibility towards PARPi (talazoparib, niraparib) combinations with topoisomerase-I inhibitors (topotecan, irinotecan). In a first-of-its-kind case, treatment of a DHG-H3G34R_ATRX patient with the brain-penetrant PARP inhibitor niraparib and topotecan resulted in significant tumor reduction.

Conclusions. Our preclinical and clinical data strongly support the further development of PARPi together with DNA damage stress-inducing treatment regimens for DHG-H3G34R_ATRX.

Key Points

- *H3G34R* mutation and loss of *ATRX* synergistically drive alternative lengthening of telomeres.
- Concomitant *H3G34R*- and *ATRX*-mutation increase DNA damage stress.
- Combination of PARP and topoisomerase inhibitors is effective against DHG-H3G34R-*ATRX*.

Graphical abstract



Importance of the Study

In this study, we identify DHG-H3G34R-*ATRX* as an ALT-driven tumor. Our analyses in iPSCs unravel that *H3G34R* mutation and *ATRX* loss synergistically and dominantly drive replicative immortality through ALT initiation. By exacerbating the resulting intrinsic DNA damage stress, a combination of PARP inhibitors with topoisomerase or DNA repair-targeting compounds

exerts synergistic activity specifically against DHG-H3G34R-*ATRX*. Accordingly, our study provides first-of-its-kind clinical evidence that this rational combination therapy can be translated into improved management of DHG-H3G34R-*ATRX* patients, struggling with an aggressive and high-risk brain tumor type.

Molecular profiling studies have disentangled distinct subtypes within pediatric high-grade glioma (pHGG). Epigenetic perturbations caused by mutations in *H3F3A*

(histone H3.3, H3), including H3K27M or H3G34R/V, are enriched in tumors of children, as well as adolescents and young adults (AYAs), respectively, suggesting distinct

modes of oncogenesis.^{1,2} While H3K27M mutations occur in younger patients and in midline/pontine locations (diffuse midline glioma, H3 K27-altered, DMG-H3K27M), H3G34R/V mutations are typically found in AYAs in the cerebral hemispheres.¹ Patients suffering from diffuse hemispheric glioma, H3 G34-mutant (DHG-H3G34) exhibit poor therapy response and prognosis.^{1,3} Therefore, the development of effective treatment options remains utterly important.

Unlimited replicative potential preventing critical telomere shortening is a key characteristic of aggressive glioma, achieved by either reactivation of telomerase reverse transcriptase (TERT) or alternative lengthening of telomeres (ALT).^{4,5} Reactivation of telomerase induced via *TERT* promoter (p*TERT*) mutations is a predictor of negative prognosis in HGG.^{6–8} Interestingly, *H3G34R* mutations frequently coexist with loss of alpha-thalassemia/mental retardation syndrome X-linked protein (ATRAX),^{1,9} functionally associated with ALT.¹⁰ However, the molecular mechanisms and cellular effects of *H3G34R* and *ATRAX* co-mutation (*H3G34R_ATRX*) and potential interaction with telomere stabilization remain elusive.

Recent sequencing analyses across various pediatric cancer types have identified mutations in the double-strand break (DSB) repair pathway, involving *breast cancer genes 1/2* (*BRCA1/2*) as potentially targetable events in brain cancers.¹¹ These findings support the role of defective DNA repair, so-called “BRCAness,” in pHGG, suggesting poly-ADP-ribose polymerase inhibitors (PARPis) as a feasible therapeutic option. PARP is a central protein in both single-strand break (SSB) and DSB repair. Its inhibition is one mainstay in targeted treatment of breast, ovarian, and prostate tumors with impaired homologous recombination (HR) repair.^{12,13} Previous work has demonstrated that loss of ATRAX in preclinical HGG models is associated with enhanced sensitivity towards DSB-inducing drugs.¹⁴ Additionally, DHG-H3G34 has been recently associated with DNA repair deficiencies.¹⁵ However, the interplay of *H3G34R_ATRX* and DNA repair remains incompletely understood.

In this study, we uncover synergistic priming of ALT by a distinct cooperative effect of *H3G34R_ATRX* in DHG (DHG-*H3G34R_ATRX*) primary patient-derived tumor cells, as well as in human induced pluripotent stem cells (iPSCs). With respect to DHG-*H3G34R_ATRX* therapy, enhanced DNA repair dependency causes synthetic lethality of PARPi combinations with DNA-damaging agents or DNA repair inhibitors. The clinical relevance was validated by a significant response of a DHG-*H3G34R_ATRX* patient to this combination treatment approach.

Materials and Methods

For detailed protocols see [Supplementary Methods](#).

Ethics Approval and Consent to Participate

The study was approved by the institutional review board of the Medical University of Vienna (MUV, EK Nr.

1244/2016). Informed consent for study participation was obtained from patients and/or their legal representatives.

Cell Culture of Patient-Derived Cell Models and Human iPSCs

Primary tumor cell models were established from fresh tissue biopsies obtained from surgical intervention. Feeder-free iPSCs 176/1 were reprogrammed from fibroblasts of healthy donors.

Generation of ATRX-Knockout and H3G34R-Overexpressing iPSCs

Plasmids¹⁶ were modified to include genome-integrable fluorescent marker cassettes to control successful plasmid delivery through the Sleeping Beauty-transposase system (pCAG-SB100X). For *ATRAX*-mutation constructs, an IRDR-LCAG-GFP-IRDR-R sequence was cloned into the CRISPR-Cas9 vector pX330-U6-Chimeric_BB-CBh-hSpCas9 (Addgene, 42230) containing *ATRAX*-targeting gRNAs as described.¹⁶ For overexpression constructs, IRDR-LCAG-X-IRDR-R (X = H3.3 WT or H3G34R) plasmids were modified to co-express a dTomato fluorophore. An overview of the constructs and experimental layouts is given in [Supplementary Figures 1 A and B](#).

Cell Viability Assays and Analysis of Drug Effects

Cells were seeded in 96-well plates and exposed to the indicated drugs for 72 hours. Viability was measured by CellTiter-Glo assay (Promega). CalcuSyn software (Biosoft) was applied to evaluate drug combination effects,¹⁷ expressed as combination indices (CI): values <0.9 = synergism; 0.9 to 1.1 = additive effects; >1.1 = antagonism.

3D Tumoroid Assay

Cells were seeded in non-adherent U-bottom-shaped 384-well plates. After 24 hours, drugs were added for 72 hours. Thereafter, the medium was refreshed for another 72 hours of drug incubation. Sphere growth was monitored with an Incucyte system (Sartorius) and tumoroid viability was measured using CellTiter-Glo 3D.

Western Blot

Cells were seeded in 6-well plates and exposed to drug treatment for 24 hours. Protein extracts were prepared and Western blots performed as published.¹⁸ [Supplementary Table 1](#) lists all antibodies used.

Immunostaining Procedures

For immunohistochemistry (IHC), paraffin-embedded tumor sections were deparaffinized and rehydrated. Blocking and antigen retrieval were followed by antibody incubation ([Supplementary Table 1](#)) and detection using

the UltraVision LP detection system (Thermo Fisher). Stainings were quantified using ImageJ (Fiji) or the histomorphometric software HALO (Indica Labs).

For multiplexed immunofluorescence analysis, tumor sections were processed using the Opal Polaris 7 Color Manual IHC Detection Kit (Akoya Biosciences) and scanned at the Phenolmager Fusion (Akoya Biosciences). Image analysis was carried out by inForm analysis software (Akoya Biosciences). [Supplementary Table 1](#) lists all antibodies used.

For in vitro experiments, cells were seeded on 10-well polystyrene cell culture slides (CELLVIEW, 543979, Greiner bio-one) and treated for 24 hours. Pre-extraction and staining with phospho-histone H2A.X and Rad51 antibodies were performed as described in [Supplementary Materials](#). Photographs were taken on the IXplore Spinning Disk Confocal (Olympus), and pH2AX/Rad51 foci were quantified using scanR (Olympus) software.

Flow Cytometry for Cell-Cycle Analysis

Cells were seeded in 6-well plates and exposed to drug treatment for 24 hours. Afterward, cells were stained with propidium iodide and analyzed on an LSR Fortessa flow cytometer (BD Biosciences) using the 610/20 bandpass emission filter. Analysis was performed with FlowJo v10.06 (BD Biosciences; for gating strategy see [Supplementary Figure 2](#)).

Analysis of Telomere-Maintenance Mechanisms

HCT116/SKOV-3 and Saos-2/U2OS cells represented TERT-positive and ALT-positive controls, respectively.

Telomerase activity was analyzed by Telomerase Repeat Amplification Protocol (TRAP-assay) as previously described.¹⁹

Telomere lengths were detected on metaphase chromosome preparations following standard protocols, applying the Telomere PNA FISH Kit/FITC (K5325, Dako). Images were captured with a DMRXA fluorescence microscope (Leica).

For detection of c-circles 32 ng of DNA were used. C-circle assay (CCA) was performed using telomeric qPCR as previously described.^{20,21}

Sequencing Experiments

Whole-exome sequencing (WES) of patient-derived cells was done on NovaSeq 6000 (Illumina) using 100 bp paired-end chemistry. Data were analyzed by alignment to the hg19 reference genome using Burrows-Wheeler Aligner (BWA MEM) and with Picard (deduplication). Variants were called with GATK Haplotype Caller and annotated with ANNOVAR.

Bulk RNA-sequencing of iPSCs was done on Illumina NovaSeq X. Reads were analyzed using genome and gene annotation for the GRCh38/hg38 assembly obtained from Homo sapiens Ensembl release 102. Reads were aligned using star v2.6.0c, processed using collapse_UMI_bam (Lexogen), and counted with featureCounts (subread v1.6.2) using strand-specific read counting (-s 1). Downstream analyses were performed in R statistical environment v4.2.0.

Irradiation Experiments

Cells seeded in 24-well plates were exposed to drug treatment combined with irradiation (2 Gy) using Maxishot (Yxlon International GmbH). After a 7-day recovery, cell clones were stained with crystal violet and quantified for color absorbance/well with the Tecan Infinite 200Pro.

In Vivo Experiments

DHG-H3G34R_ATRX patient-derived tumor cells were used for chorioallantoic membrane (CAM) and orthotopic xenografts. CAM assays were performed before hatching, and thus, did not require ethical approval.²² Briefly, tumor cells were inoculated on embryo development day (EDD) 9 onto the CAM. On EDD10, drug treatment was initiated by pipetting the drugs onto the CAM, and repeated on EDD12, EDD14, and EDD16. On EDD17, tumor volumes were measured, and tumors were embedded for IHC analysis.

For the orthotopic animal experiment, near-infrared fluorescent protein (iRFP) labeled tumor cells were implanted via the postglenoid foramen into the brains of NSG mice. Upon detection of tumor development, mice were randomized to control, topotecan monotherapy (0.5 mg/kg/day i.v.) or combination therapy with niraparib (5 mg/kg/day p.o.). The experiment was conducted in an AAALAC-accredited animal facility under permit G23/036 of the Regierungspräsidium Freiburg im Breisgau, Germany.

Case Study

Data was retrieved from medical records at the General Hospital/MUV. The diagnosis was confirmed by methylation analysis and next-generation sequencing. Treatment with niraparib and topotecan was based on off-label use under close clinical and laboratory monitoring. MRI, magnetic resonance spectroscopy (MRS), as well as PET imaging, were conducted per clinician discretion to monitor tumor response and further analyzed by central radiological assessment according to response assessment in pediatric neuro-oncology criteria.

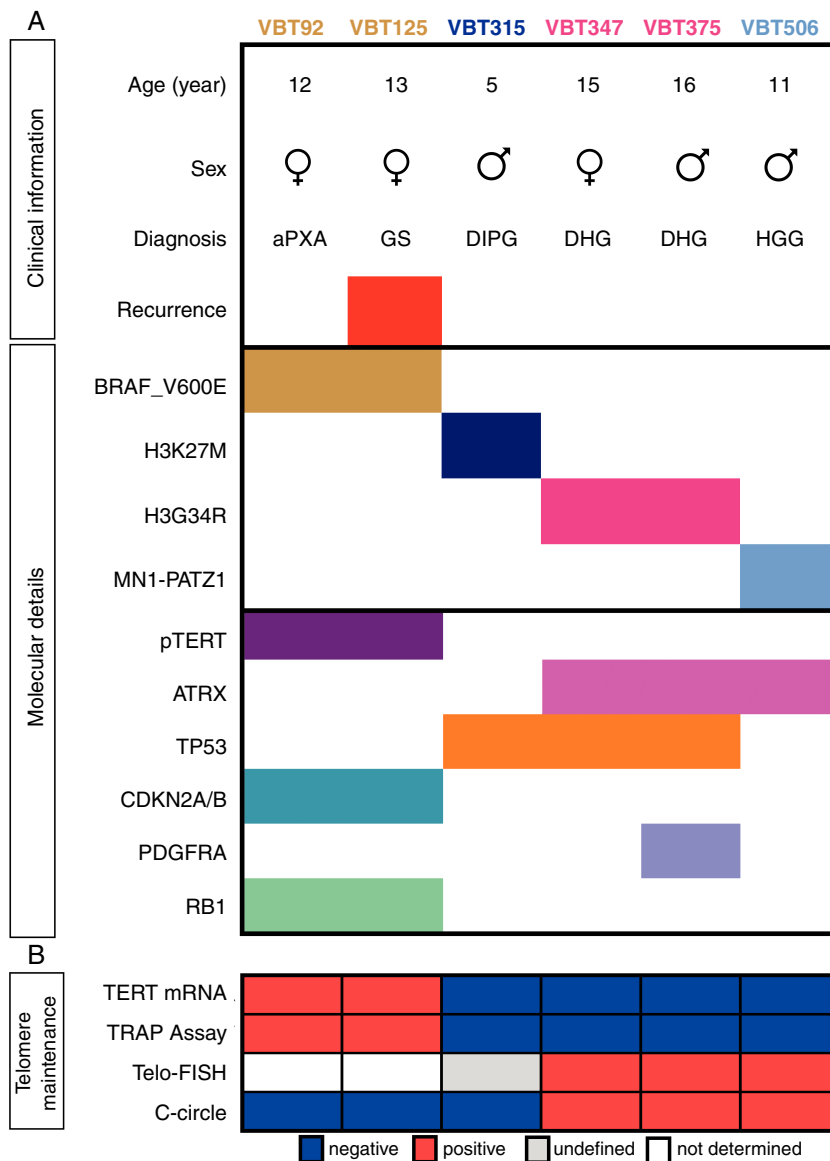
Statistical Analysis

Appropriate statistical tests are described in the respective figure legends. The NEJM *P*-value style was chosen for all analyses. *P*-values <0.05 were considered statistically significant. **P*<0.05; ***P*<0.01; ****P*<0.001.

Results

H3G34R_ATRX Co-mutations Segregate With ALT-Phenotype in Primary Patient-Derived and Human iPSC Models

To investigate *H3G34R_ATRX* co-mutations, we compiled a substantial panel of pHGG cell lines (*n*=6) including 2 previously published TERT-driven models⁷ as non-*H3G34R/non-ATRX*-mutated controls ([Figure 1A](#)). For the



ATRX-mutated models are associated with ALT-phenotype. **(A)** Overview of the patient-derived pHGG cell line panel providing clinical information and molecular details. Mutations: *BRAF_V600E*, *H3K27M*, *H3G34R*, *TERT* promoter (*pTERT*), *ATRX*, *TP53*, *PDGFRA*, *RB1*. Fusion: *MN1::PATZ1*. Chromosomal loss: *CDKN2A*. aPXA: anaplastic pleomorphic xanthoastrocytoma, GS: gliosarcoma, DIPG: diffuse intrinsic pontine glioma, DHG: diffuse hemispheric glioma, HGG: high-grade glioma. **(B)** Overview of the telomere-maintenance mechanisms examined in the indicated patient-derived pHGG cell models. Results from the following assays are depicted: *TERT* mRNA levels analyzed by quantitative real-time PCR (*TERT* mRNA), telomerase activity measured with TRAP-assay, and presence of ALT assessed with Telo-FISH and c-circle assay.

characterization of the mutational landscape, we performed WES. All 3 H3-mutated models harbored *TP53* (tumor protein p53) mutations. Both H3G34R models demonstrated an *ATRX* mutation, while the 2 B-Raf proto-oncogene (*BRAF*) *V600E*-mutant models harbored *pTERT* mutations as described.⁷ In line with our cell models, a comprehensive cohort compiling own and published data ($n = 35$) confirmed concomitant *ATRX* and *TP53* mutations in 91% of H3G34R tumors (Supplementary Figure 3A). These findings are in consonance with a previously published meta-analysis, indicating frequent co-mutations of *ATRX* and *TP53* in DHG-H3G34 tumors.²³ The *ATRX* mutations of our

DHG-H3G34R models were located in the ATP-binding helicase domain, but upstream of the DAXX-binding domain in the *MN1::PATZ1* model (Supplementary Figure 3B). Despite elevated *ATRX* mRNA expression in 2 models, all 3 *ATRX*-mutated cell lines exhibited loss of basal *ATRX* protein expression (Supplementary Figure 3C, D). This was further reflected by decreased H3 trimethylation at lysine 9 (H3K9me3) in all *ATRX*-mutated models, consistent with the well-described histone-modifying function of *ATRX*^{24–26} (Supplementary Figure 3D).

To evaluate the telomere-maintenance mechanisms of our pHGG, we employed different telomerase- and

ALT-specific assays (Figure 1B). Elevated *TERT* expression and telomerase activity were only found in *BRAF_V600E* and *pTERT* co-mutated cells (VBT92, VBT125; Figures 1B, 2A, B, Supplementary Figure 4).⁷ In contrast, *ATRX*-mutant models (VBT347, VBT375, and VBT506) showed distinct ultra-bright intranuclear telomere spots in Telo-FISH, heterogeneous length of telomeres, and the presence of extrachromosomal circular telomere repeats (Figure 2C, Supplementary Figure 5), all indicating an ALT phenotype. Together, these results suggest that TERT reactivation and ALT are mutually exclusive in pHGG. For the DMG-H3K27M cell model VBT315, no clear telomere-maintenance mechanism was identified, albeit a heterogeneous appearance of telomeres suggesting ALT (Supplementary Figure 5). Interestingly, the ALT-phenotype was more pronounced in DHG-G34R_ATRX models (VBT347, VBT375) than in the *ATRX*-mutated *MN1::PATZ1*-fused model (VBT506). Consequently, we hypothesized that *H3G34R_ATRX* co-mutations synergistically induce ALT.

To dissect the role of H3G34R_ATRX in telomere stabilization in a non-malignant background, we assessed the presence of telomeric c-circles in human iPSCs overexpressing either H3.3 wild type (H3.3-WT-OE) or H3G34R (H3.3-G34R-OE) with or without *ATRX*-knockout (*ATRX*-KO). We aimed to compare H3.3-G34R-OE_ATRX-KO to H3.3-WT-OE_ATRX-KO and single *ATRX*-KO iPSCs. In agreement with previous observations,^{27,28} both single *ATRX*-KO and H3.3-WT-OE_ATRX-KO iPSCs were not viable, in contrast to H3.3-G34R-OE_ATRX-KO. This suggests an essential pro-survival function of the *H3G34R* mutation in combination with loss of *ATRX*. The H3.3-G34R-OE_ATRX-KO iPSC model showed identically decreased levels for ATRX and H3K9me3 as the patient-derived DHG-G34R_ATRX models (compare Supplementary Figure 3E). As expected, the H3.3-G34R-OE iPSC model demonstrated an upregulated expression of H3K9me3.²⁹ Resembling patient-derived DHG-H3G34R_ATRX, we detected c-circles solely in H3.3-G34R-OE_ATRX-KO iPSCs (Figure 2D). In addition, unsupervised transcriptome analysis revealed “DNA_Damage_Telomere_Stress_Induced_Senescence” as the most significantly enriched pathway in H3.3-G34R-OE_ATRX-KO iPSCs compared to H3.3-WT (NES: 1.88, p. adj: 0.04, Supplementary Figure 6A, B; Tables 3, 4). Furthermore, we found altered expression of DNA repair genes driven by H3.3-G34R-OE, including distinct downregulation of *ATRX* expression (Supplementary Figure 6C). These observations indicate that the H3G34R mutation is a prerequisite for cell survival upon *ATRX* inactivation, consequently allowing the development of an ALT phenotype at least in iPSCs (Figure 2D).

H3G34R_ATRX Alterations are Associated With Increased DNA Damage Stress in pHGG Tumors and Human iPSCs

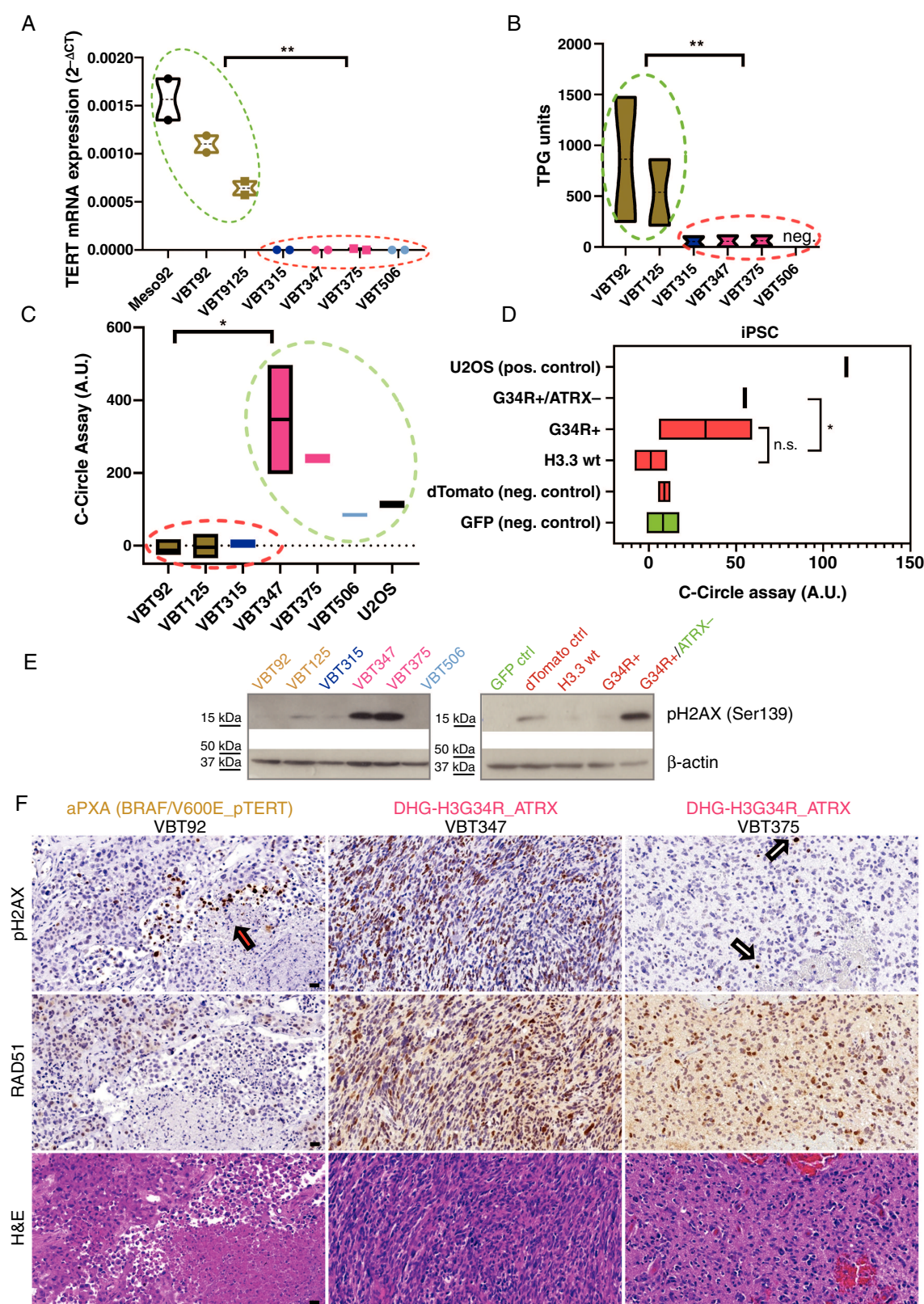
Previous studies have shown the individual impact of *ATRX* loss or *H3G34* mutations on DNA repair, but information regarding potential cooperative effects is currently lacking.^{14,15,24} Consequently, we analyzed the basal expression of DNA repair proteins in our pHGG cell line panel. Expression of PARP and respective protein PARylation

(pADPr) were detected in all models, except VBT125, which showed low expression of all DNA repair proteins investigated (Supplementary Figure 7A, B). Likewise, the *TP53*-mutant DMG-H3K27M model VBT315 showed low basal DNA repair protein expression. Concerning repair of toxic DSBs, expression of Rad50, a component of the Mre11-Rad50-Nbs1 complex operating in non-homologous end-joining,³⁰ was detected in all models except VBT125, while HR protein Rad51 expression³⁰ was only observed in VBT92 and VBT375 cells (Supplementary Figure 7A, B). It has been shown that Rad51 is not necessary for ALT telomere stabilization in U2OS, but that loss of Rad51 increases c-circle levels.³¹ Indeed, the DHG-H3G34R_ATRX model VBT347 lacking Rad51 expression showed higher amounts of c-circles than Rad51-expressing VBT375 cells (Figure 2C). Furthermore, exclusively DHG-H3G34R_ATRX models exhibited elevated basal levels of phosphorylated H2AX (pH2AX), a marker for DNA damage³² (Figure 2E). Corroborating the increased levels of pH2AX observed in DHG-H3G34R_ATRX, we detected phosphorylation of H2AX in H3G34R-OE_ATRX-KO iPSCs (Figure 2E) but no changes in the expression of other DNA repair proteins (Supplementary Figure 7C).

Since these in vitro data suggest that DNA damage stress is associated with DHG-H3G34R_ATRX, we assessed pH2AX- and Rad51-positive nuclei in fixed tissue samples of 2 DHG-H3G34R_ATRX cases (matching cell models VBT347 and VBT375) as compared to a TERT-driven aPXA tumor tissue (matching VBT92). Reflecting the cell models, the DHG-H3G34R_ATRX tissues generally showed moderate to strong vs. isolated nuclear pH2AX staining in VBT347 and VBT375, respectively (Figure 2F). Rad51 signals were overlapping with (VBT347) or even exceeding the pH2AX signals (VBT375). In contrast, no pH2AX signal was observed in vital tumor regions of the TERT-driven aPXA tissue (Supplementary Figure 8), except alongside necrotic regions (Figure 2F).

Combined Inhibition of PARP and Topoisomerase or the ATR Pathway Shows Synergistic Activity in pHGG and iPSCs With H3G34R_ATRX

Considering increased basal DNA damage in DHG-G34R_ATRX, we next investigated vulnerability towards DNA-damaging agents. All models showed intrinsic resistance against the frequently used DNA-damaging compound temozolomide (TMZ), with IC_{50} values higher than 250 μ M (a common reference value in literature; Supplementary Figure 9A).³³ Next, we determined the sensitivity towards single-agent PARP inhibition. We defined PARPi sensitivity by an IC_{50} value below 10 μ M, according to a recently published talazoparib sensitivity definition for pediatric cancers.³⁴ All models showed intrinsic resistance towards monotherapy with 7 different PARPis (Supplementary Figure 9A). Profiling of other drugs targeting DNA repair was performed according to published IC_{50} values of *BRCA*-mutated breast cancer cell lines (data were acquired from the Harvard Medical School LINCS Center, Dataset ID:20343 and Genomics of Drug Sensitivity in Cancer, Release 8.4, July 2022).^{35–37} All cell models were resistant against the DNA-crosslinking agent cisplatin



ALT parameters and DNA repair perturbations are observed in H3G34R_ATRX primary patient-derived and human iPSC models. (A) Mean mRNA expression of *TERT* in patient-derived pHG cell models ($n = 2$ biological repeats in triplicate). The mesothelioma cell line Meso92 (Pirker, Bilecz, et al. 2020) was used as a positive control. Differences in *TERT* mRNA levels between *TERT*-positive (upper circle) and *TERT*-negative (lower circle) pHG models were quantified by unpaired student's *t*-test with two-tailed distribution (** $P < .01$). (B) Results of

the TRAP-assay are presented as telomerase product generated (TPG) units ($n = 2$ biological repeats). Differences in telomerase activity between *TERT*-positive (upper circle) and *TERT*-negative (lower circle) pHGG models were quantified by unpaired student's *t*-test with a two-tailed distribution (** $P < .01$). (C) Bar graph showing relative c-circle levels of patient-derived pHGG cell lines ($n = 2$ biological repeats in triplicate). ALT-specific telomeric c-circle content was determined by qPCR following rolling circle amplification with or without Phi polymerase and quantified relative to U2OS (ALT-positive osteosarcoma cell line). Significant differences between ALT-positive (green circle) and ALT-negative (red circle) pHGG models were evaluated by One-way ANOVA with Bonferroni post-test. * $P < .05$. (D) Box plot showing relative c-circle levels of iPSCs. Telomeric c-circle content was determined as described in (C); $n = 2$ biological repeats in triplicate). Significance levels were evaluated by unpaired student's *t*-test with a two-tailed distribution. * $P < .05$. (E) Western blot analyses showing phosphorylation of the DNA damage marker H2AX in the patient-derived pHGG cell line panel (upper panels) and iPSCs (lower panels). β -actin served as a loading control. (F) Immunohistochemical staining of pH2AX and Rad51 in tissue sections of one *BRAF_V600E* and *pTERT* co-mutated aPXA tumor (matching cell model VBT92) and 2 DHG-H3G34R_ATRX tumors (matching cell models VBT347 and VBT375). The red arrow marks necrosis and the white arrows indicate single pH2AX-positive nuclei in the second DHG-H3G34R_ATRX tumor (VBT375). Scale bar = 20 μ m.

(Supplementary Figure 9A). Concerning topoisomerase 1 inhibitors, we detected sensitivity towards the active metabolite of irinotecan, SN-38, and topotecan in VBT125 and VBT347 (Supplementary Figure 9A). This sensitivity of the *BRAF_V600E* and *pTERT* co-mutated recurrence model VBT125 might be derived from the treatment with BRAF/MEK inhibitors, recently associated with induction of a BRCAness-like state in *BRAF*-mutated melanoma.³⁸

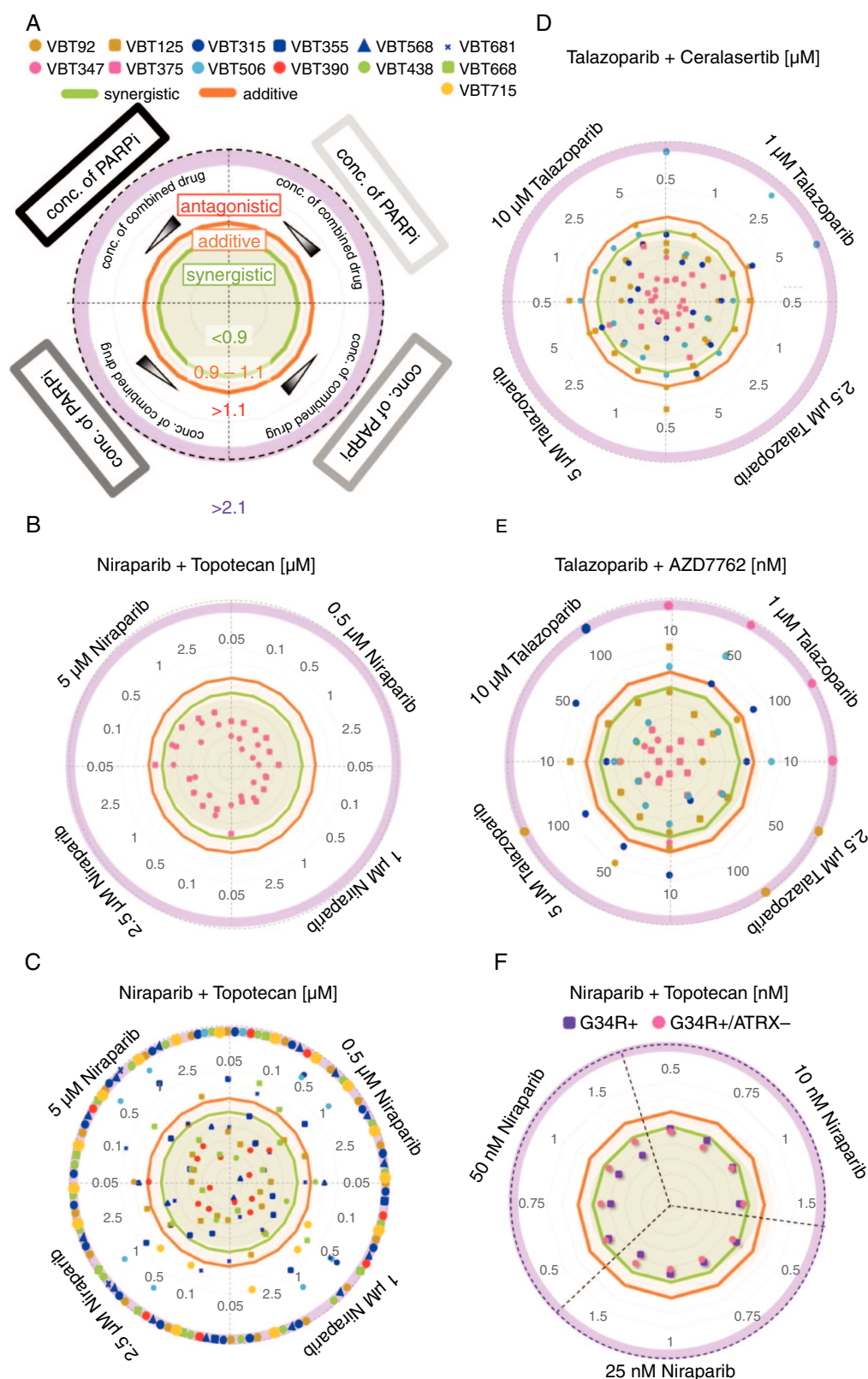
As ALT-positive cells have been shown to be sensitive towards ataxia telangiectasia and Rad3-related (ATR) inhibition,³⁹ we tested our cell line panel for ATR pathway inhibition response. Ceralasertib reduced cell viability in all models, however, independent of their ALT status (Supplementary Figure 9A). This is of particular interest with regard to the DMG-H3K27M model VBT315, which showed selective sensitivity towards ceralasertib. Inhibitors downstream of ATR, AZD-7762, and prexasertib targeting checkpoint kinases 1 and 2 (CHK1/2), markedly reduced the viability of VBT125 and VBT375 cells (Supplementary Figure 9A). Any significant impact of the solvent dimethylsulfoxid (DMSO) could be excluded, except for TMZ in the VBT506 model (Supplementary Figure 9B).

PARPis have been shown to enhance the cytotoxic activity of multiple chemotherapeutics, including topoisomerase inhibitors, alkylating, and platinum-containing agents, as well as ionizing radiation.^{12,40–46} Following the detection of increased PARylation levels in DHG-H3G34R_ATRX models, we assessed the synergistic activity of PARPis with topoisomerase or ATR pathway inhibitors (Figure 3A). We selected talazoparib, exerting pronounced PARP-trapping activity,⁴⁷ for combination studies. Synergistic effects between talazoparib and SN-38 were only observed for the SN-38-sensitive models VBT125 and VBT347 (Supplementary Figure 10). The combination of niraparib, the second most potent PARP-trapping agent,⁴⁷ with the topoisomerase inhibitor topotecan demonstrated a clear trend towards synergistic interactions for the 2 DHG-H3G34R_ATRX models (VBT347, VBT375; Figure 3B, Supplementary Figure 11, 12A), as well as VBT125 (Figure 3C, Supplementary Figure 11, 12A), whereas antagonistic effects were observed in all other models (Figure 3C, Supplementary Figure 11, 12A). We extended the cell line panel by including 7 additional models (3 DMG-H3K27M models, one H3-WT HGG, one infant-type hemispheric glioma, and 2 *NF1*-altered models; Supplementary Figure 12A), but no comparable specific synergistic interactions for niraparib combined with topotecan were detected (Figure 3C, Supplementary Figure 12A, B). Concomitant

targeting of PARP and ATR or CHK1/2 exerted strong synergistic effects for all 3 combinations tested selectively in DHG-H3G34R_ATRX (Figure 3D, E, Supplementary Figure 13, 14). Combined inhibition of PARP and ATR was the most effective approach for the DMG-H3K27M model VBT315 (Supplementary Figure 13). We confirmed these observed synergies of niraparib with topotecan or talazoparib in combination with ATR- or CHK1/2-inhibitors in 2 additional DHG-H3G34R_ATRX models (*TP53* mutant; Supplementary Figure 15A, B). To investigate the effects of combined niraparib with topotecan treatment on multicellular tumor composition, we conducted 3D tumoroid assays of DHG-H3G34R_ATRX cell models (VBT347, VBT375) and observed reduced viability, especially concerning VBT375 tumor spheroids upon combination treatment (Supplementary Figure 16A, B, C). Concerning iPSCs, deletion of *ATRX* in H3G34R-OE resulted in a more predominant synergistic pattern of niraparib and topotecan, corroborating the observations in patient-derived tumor models (Figure 3F). Together, we identify that DHG-H3G34R_ATRX models are highly susceptible to PARPi-based combinations with either topoisomerase- or ATR-inhibitors, opening novel therapeutic strategies.

Combined Inhibition of PARP and Topoisomerase-I Causes Replication Stress and Persistent DNA Damage in ATRX-Mutated DHG-H3G34R Models

Next, the cellular effects of PARPi combinations on cell-cycle distribution were analyzed. We observed induction of an S-phase arrest by topotecan in DHG-H3G34R_ATRX (VBT347) but not aPXA (VBT92) cells, albeit this effect was reduced when niraparib was added (Figure 4A, Supplementary Figure 17A). In addition, we tested the combination of talazoparib with SN-38, resulting in an increased S-phase arrest in DHG-H3G34R_ATRX (VBT375) but not in aPXA cells (VBT92, Supplementary Figure 17B). Expression of PARP and PARylation levels were selectively reduced by niraparib in DHG-H3G34R_ATRX cells (VBT347; Figure 4B). We further detected increased levels of pH2AX, that were highest in the combination of topotecan with high-dose niraparib and more pronounced in DHG-H3G34R_ATRX (VBT347) compared to aPXA (VBT92) cells. Furthermore, expression of the DNA damage signaling protein ATR, involved in stalled replication forks signaling,⁴⁸ and activation/upregulation of



Specific synergistic activity of PARPi combination therapies in H3G34R_ATRX patient-derived cell and iPSC models. **(A)** Schematic representation of a circular synergy dot-plot showing mean combination indices (CI) values. The small inner circle depicts synergistic CI values (<0.9). The middle circle represents additive CI values ($0.9-1.1$) and CI values >1.1 are considered antagonistic (white circle). Antagonistic CI values >2.1 are collected in the light violet circle boundary. CI values were determined with CalcuSyn. CI <0.9 , synergism; CI $=0.9-1.1$, additive effects; or CI >1.1 , antagonism. Each quarter represents one concentration of the PARPi and within each quarter increasing doses of the

combined drug are shown. (B-E) Circular synergy dot-plots for niraparib in combination with topotecan (B-C), talazoparib with either ceralasertib (D) or AZD-7762 (E) are presented. Mean CI values based on the cell viability data of patient-derived pHGG cell models were calculated from $n = 3$ independent experiments. In case of VBT125 and VBT347 topotecan concentrations are 0.005; 0.025; 0.05 and 0.075 μM . (F) Circular synergy dot-plots for niraparib in combination with topotecan in H3.3 G34R OE and H3.3 G34R OE_ *ATRX* KO iPSCs. Mean CI values based on the cell viability data of iPSC models were calculated from $n = 3$ independent experiments.

p53 or p21 were missing in DHG-G34R_ *ATRX* (VBT347) compared to aPXA (VBT92) cells (Figure 4B). We further investigated the effect of combined niraparib with topotecan in our iPSC models. Unsupervised transcriptome analysis revealed the reactome pathway “Response_of_EIF2AK1_HRI_to_heme_deficiency” (heme-regulated inhibitor (HRI)-induced protein translation inhibition), related to cell death induction,^{49,50} as top significantly enriched pathway in H3.3-G34R-OE_ *ATRX*-KO iPSCs upon combination treatment compared to H3.3-WT (NES: 2.07, P_{adj} : 0.005; Supplementary Figure 18A, B, Tables 5 and 6). On the protein level, we found massively increased cleavage of PARP, accompanied by an increase in pH2AX protein levels specifically in H3.3-G34R-OE_ *ATRX*-KO iPSCs (Supplementary Figure 18C). Furthermore, we observed massive downregulation of protein PARylation by H3G34R overexpression (Supplementary Figure 18C). Corroborating these results, we detected increased pH2AX and decreased Rad51 foci in untreated DHG-G34R_ *ATRX* (VBT347) cells when compared to aPXA (VBT92, compare Figures 2E and 5A and B, Supplementary Figures 7A and 19). Moreover, VBT92 cells showed a significant surplus of Rad51 (Supplementary Figure 19). While treatment increased pH2AX foci formation in both cell models, only in VBT92 cells pH2AX foci were outnumbered by Rad51 foci (Figure 5A and B, Supplementary Figure 19). Taken together, concomitant treatment with niraparib and topotecan induces increased replication stress and impairs the repair of DNA damage causing persistent DSB in DHG-H3G34R_ *ATRX* cells.

Anti-Cancer Effect of Niraparib and Topotecan Therapy in Vivo

In order to study the anti-cancer effect of niraparib combined with topotecan in a multicellular model, we conducted CAM assays with the DHG-H3G34R_ *ATRX* model VBT347. However, no significant changes in tumor volume were found between all groups (Supplementary Figure 20A). Nevertheless, we observed a significantly reduced expression of the human glial marker GFAP, indicating reduced tumor cell density (Supplementary Figure 20 B and C). Furthermore, we investigated the combination treatment in a DHG-H3G34R_ *ATRX* orthotopic PDX model (Supplementary Figure 21A). Mice treated with the combination showed a trend toward a delayed onset of accelerated tumor growth and longer survival (Supplementary Figure 21 B, C, and D). Due to the model-inherent highly variable time to tumor progression in all experimental groups, these data did not reach statistical significance. Nevertheless, histo-pathological analysis revealed a significantly reduced tumor cell density in the combination group (Figure 5C, Supplementary Figure 22). As a next step, we sought to interrogate the impact of therapy on

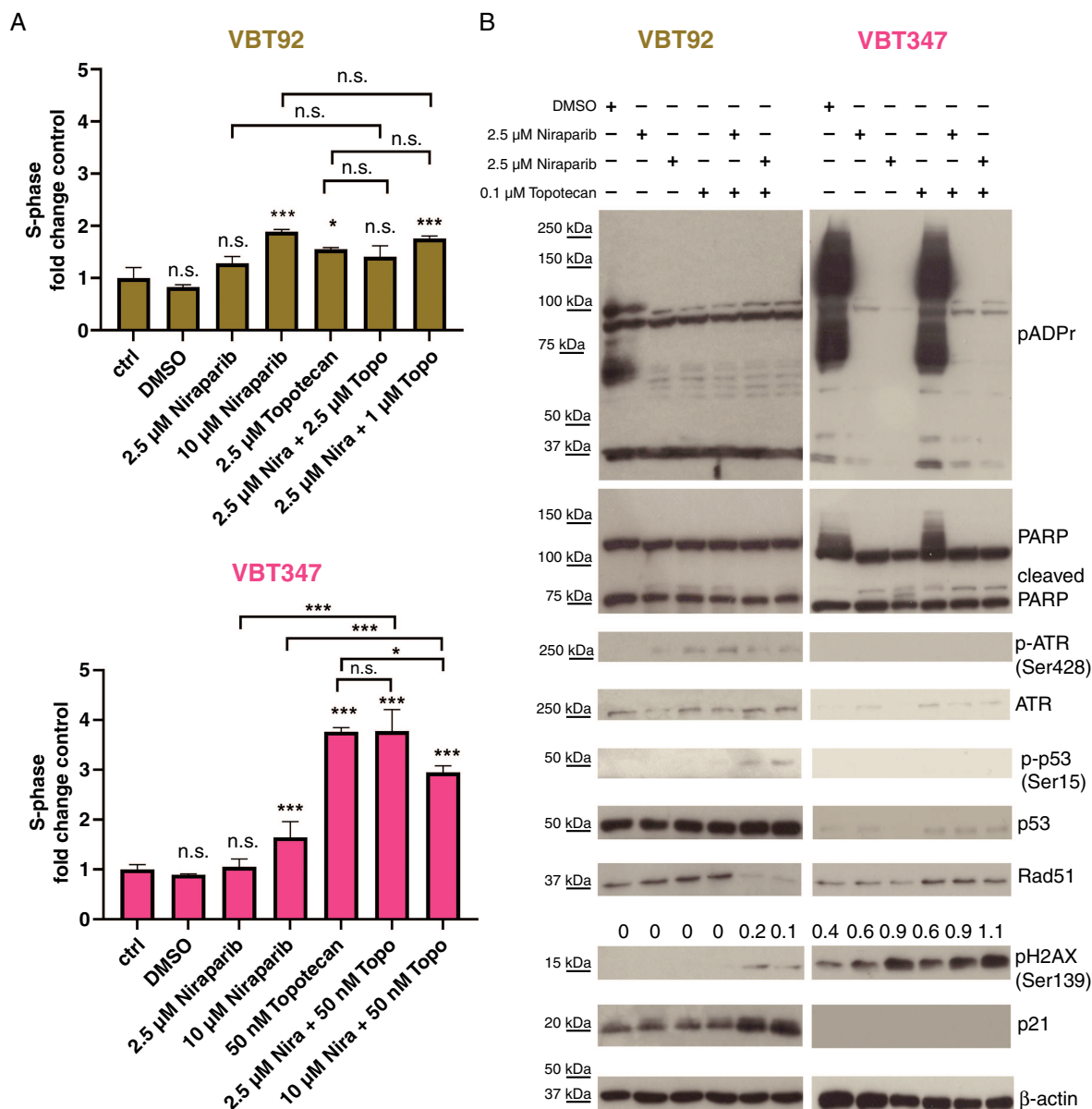
intra-tumoral heterogeneity and cell states. Multiplexed immunofluorescence phenotyping revealed a significant decrease of stemness (nestin) and protein translation (p4EBP1) markers in the combination therapy arm (Figure 5D, E, Supplementary Figure 23 A and B). *Vice versa*, markers for interneuronal maturation (TUBB3, DCX) were significantly increased in the treatment groups (Supplementary Figure 23B).

Treatment of a Radiotherapy-Resistant DHG-H3G34R_ *ATRX* Case With Concomitant Inhibition of PARP and Topoisomerase

Based on our preclinical findings, we decided to treat a 15-year-old girl (harboring the VBT347 tumor) diagnosed with *TP53*-mutated DHG-H3G34R_ *ATRX* with a PARPi and a topoisomerase inhibitor, after failed first-line therapy. Initially, the patient presented due to tonic-clonic seizure and received near-total tumor resection (Figure 6A), followed by local radiotherapy with 60 Gy and concomitant TMZ (75 mg/m²). MRI 4 weeks after completion of radiotherapy showed a local tumor progression within the radiation field (Figure 6B). MRS showed increased choline/NAA ratios, further favoring progression over pseudoprogression or radiation necrosis (Figure 6B, Supplementary Figure 24). Radiotherapy resistance was confirmed in the respective VBT347 cell model. Combination with niraparib reversed radioresistance in VBT347 but not in VBT92 cells (Supplementary Figure 25). Considering the early in-field recurrence, treatment with niraparib and concomitant topotecan was initiated 7 weeks after radiotherapy completion. Niraparib was initiated at 300 mg/day with topotecan 1.5 mg/m² on days 1–3 in 21-day cycles and adapted following hematological side effects (details in Supplementary Methods). Regular follow-up with MRI showed a continuous decrease of tumor size (representative MRI after 8 months, Figure 6C) and partial tumor response. In general, treatment was well tolerated, with good quality of life. In total, 13 cycles were administered. However, thirteen months after combination treatment initiation, MRI revealed progressive disease with a new tumor manifestation precentral, in the right thalamus (Figure 6D).

Discussion

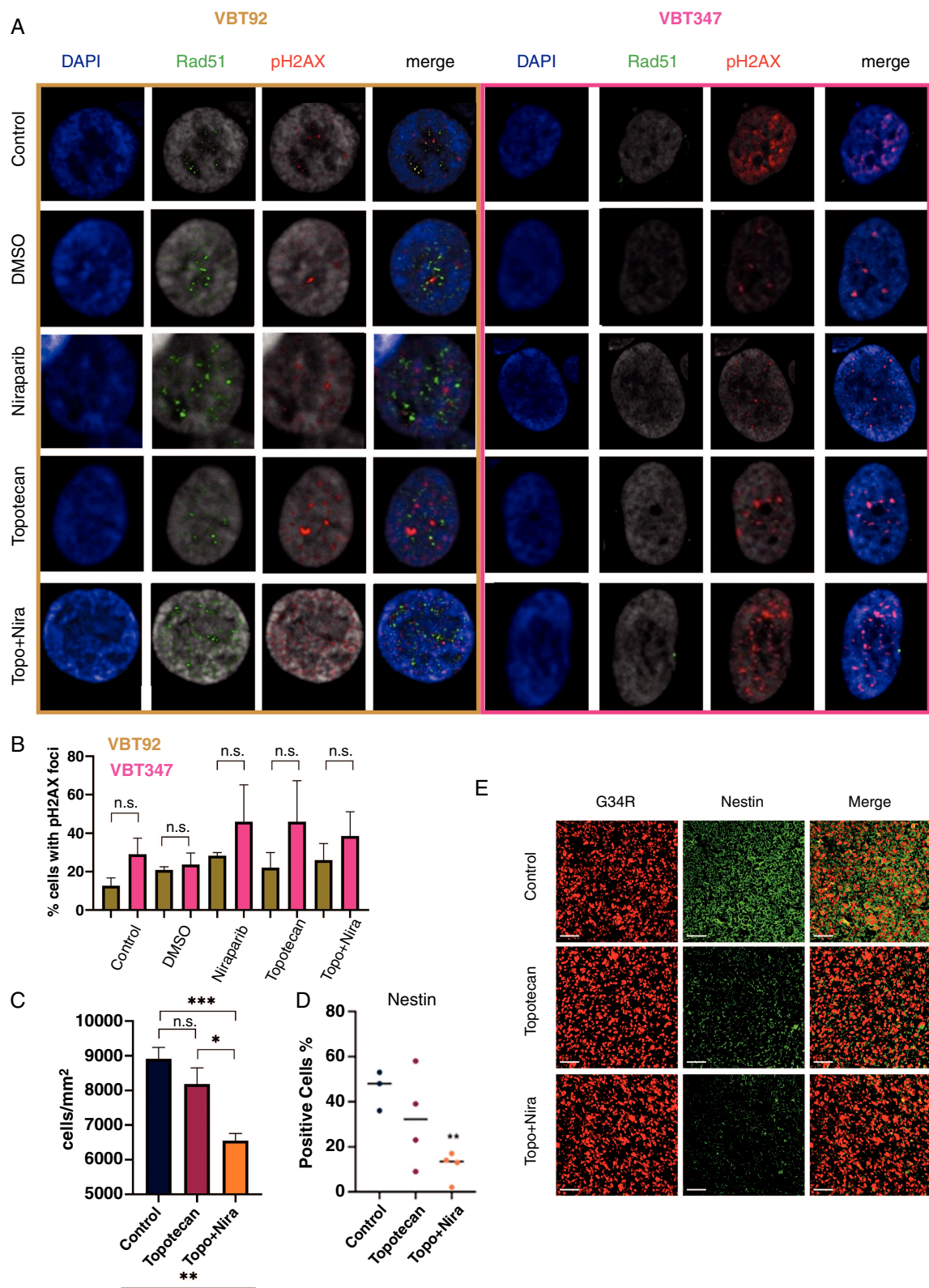
Despite advances in the molecular understanding of the underlying biology, treatment of pHGG remains challenging. While the epigenetic effects of the key pHGG H3 mutations *H3K27M* or *H3G34R/V* have been extensively studied, translation into clinically approved therapeutic regimens is still missing. Here, we demonstrate that



Combined inhibition of PARP and topoisomerase causes cell-cycle perturbations and exacerbates DNA damage stress in DHG-H3G34R_ATRX models. **(A)** The impact of niraparib combined with topotecan at the indicated concentrations for 24 hours on the proportion of cells in S-phase of the cell cycle of VBT92 and VBT347 cells was analyzed with PI-based flow cytometry. Data of $n = 3$ independent experiments in triplicate are given and normalized to the respective untreated controls. Results are represented as mean $\pm$ SD of the respective untreated control. Significance levels between untreated and treated samples, as well as single-drugs and combinations, were evaluated by One-way ANOVA with Bonferroni post-test. * $P < 0.05$; *** $P < 0.001$; n. s. not significant. **(B)** Western blot analyses of VBT92 and VBT347 cell models treated for 24 hours with niraparib alone or in combination with topotecan with the indicated concentrations. Expression, cleavage, and phosphorylation of the indicated proteins involved in DNA repair are shown. β -actin served as a loading control. Densitometric quantification insets show phosphorylation of H2AX normalized to β -actin.

DHG-H3G34R tumors with *TP53* mutation and loss of ATRX constitutively exert ALT-driven telomere maintenance. ALT is associated with the collapse of the ATRX-DAXX histone chaperone complex,²⁶ and mutations of *ATRX* and *DAXX* occur mutually exclusive.²⁶ We also found *ATRX* loss in

one *MN1::PATZ1*-fused case lacking *H3F3A* or *TP53* alterations, exhibiting signs of ALT activation, however, less pronounced as observed for the DHG-H3G34R_ATRX models. This fits a previous study in mouse embryonic stem cells (mESCs) describing the combined loss of *ATRX*,



The combination of niraparib and topotecan drives DNA damage and stemness marker loss in DHG-H3G34R_ATRX models. (A) Immunofluorescence staining of pH2AX (DNA damage, right) and Rad51 (DNA repair, middle) in VBT92 (*pTERT*-mutated aPXA) and VBT347 (DHG-H3G34R_ATRX) DAPI-stained nuclei (left). Cells were untreated (control) or treated with vehicle (DMSO), 10 μ M niraparib or/and 0.1 μ M topotecan for 24 h. Images were captured on an Olympus spinning disk confocal microscope (40 $\times$ objective). Representative images of one experiment are shown. (B) Quantification of pH2AX foci per nucleus of VBT92 and VBT347 cells as depicted in (A), represented as a percentage of foci-positive cells. A minimum of 1500 cells per condition were quantified and analyzed with ScanR ($n = 3$ independent experiments). Error bars represent

mean $\pm$ SEM. (C) Tumor cell density was measured in DHG-H3G34R_ATRX PDX brain tissues of control, topotecan monotherapy, or topotecan combination therapy with niraparib-treated mice. One sample of the combination treatment group showed no tumor in the brain parenchyma, except alongside the injection channel, and was therefore excluded from this analysis. Statistical significance was calculated with unpaired two-tailed student's *t*-test and two-way ANOVA with Dunnett post-test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; ns not significant. (D) Quantification of nestin (stemness)-positive cells. 2.2 million H3G34R-positive tumor cells were phenotyped. Profiling was performed on all samples containing bulk tumors. Statistical significance between untreated and treated was evaluated by unpaired student's *t*-test. (E) Representative immunofluorescence images for the stemness marker (nestin). Scale bar = 100 μ m.

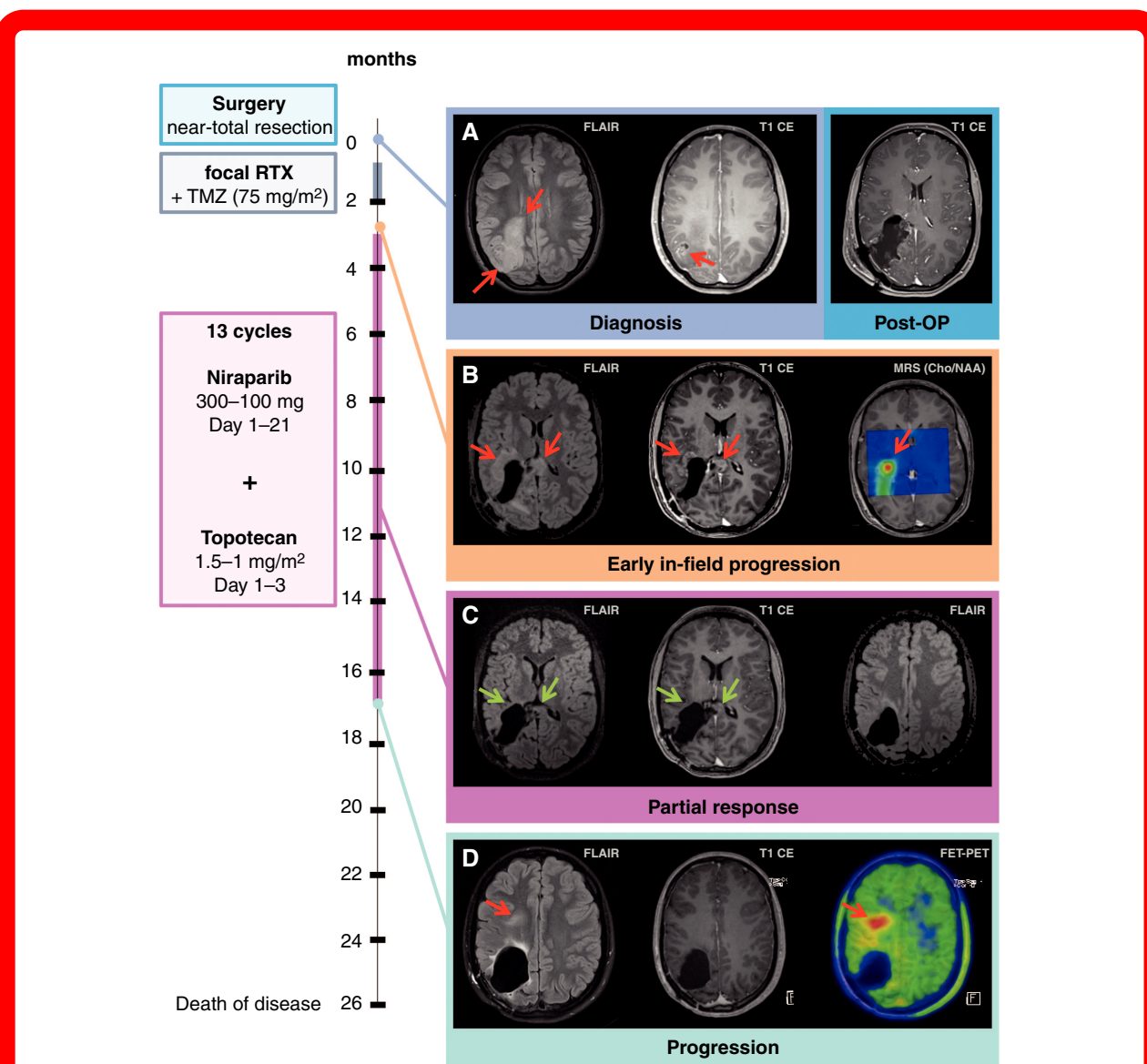
p53, and *TERT*, together with either *H3G34R* or *IDH* mutation as necessary for full ALT initiation.⁵¹ By genetic modification of human iPSC models, we show for the first time that *H3G34R_ATRX* directly cooperates to induce a viable cellular ALT phenotype. This is of particular interest, as H3 and ATRX cooperatively maintain chromatin structures. H3 is regulated by methylation of diverse lysine residues, subsequently influencing chromatin accessibility (hetero- or euchromatin) and gene expression.⁵² The *H3G34R* mutation exerts a dual epigenetic function, as it increases both H3K9me3 (repressive mark) and H3K36me3 (activating mark), reflected also in our transcriptome analyses of iPSC models.^{29,52} ATRX is well known to preferably deposit H3 WT to H3K9me3-enriched (silenced) regions, maintaining pericentromeric heterochromatin and telomeres in a transcriptionally inactive state.^{25,26} Moreover, in a previous study, the transcriptionally active 3' exonic regions of zinc finger genes (ZNFs) with elevated levels of H3K36me3 and H3K9me3 have been identified as binding targets for ATRX.⁵³ Thus, the epigenetic perturbations caused by *H3G34R* may interact with ATRX recruitment to specific regulatory chromatin regions. Indeed, we found that the elevation of H3K9me3 by *H3G34R* expression was reversed upon additional knockout of *ATRX*.

Interestingly, the *H3G34R* mutation causes perturbed DNA damage repair in yeast cells as well as reduced proliferation in eukaryotic cells.^{15,54} However, overexpression of *H3G34R* in iPSCs in our study did neither induce enhanced phosphorylation of H2AX nor *p53* overexpression and was compatible with cell viability. In contrast, knockout of *ATRX* in a wild-type *H3F3A* background was not viable, which is in agreement with the above-mentioned mESC study.⁵¹ Strikingly, the *H3G34R* mutation caused decreased *ATRX* expression and was necessary to allow survival of *ATRX*-inactivated iPSCs. Thus, *H3G34R_ATRX* appears to be essential for immortalization by activation of ALT in DHG-H3G34R tumors. With regard to DHG-H3G34R, it is important to mention that, in line with published data,^{1,2,55} the 2 cell line-forming DHG-H3G34R cases in our study also harbor *TP53* mutations in addition to *ATRX* loss. This strongly suggests that for the full process of tumorigenesis, ALT must be accompanied by an impaired DNA damage response, allowing tumor cells to survive enhanced DNA damage stress associated with the ALT phenotype. Regarding iPSCs, it needs to be considered, that analyses were performed in a telomerase-positive stem cell background. ALT induction by *H3G34R_ATRX* in the presence of active telomerase proposes ALT activation to be an autonomous process, not requiring selection due to telomere crisis. Comparable observations for ALT activation have recently been reported by co-mutation of *ATRX*, *TP53*, *RB*, and *CDKN2A*.⁵⁶

H3, a major histone variant necessary for DNA repair, is deposited to sites of UV-induced damage and DSB.⁵⁷ Consequently, *H3G34R* mutation and *ATRX* loss have individually been shown to interfere with DNA damage response in HGG,^{15,24} but the cooperative effect has not been studied yet. Recently, increased tolerance of ALT-driven pHGG to DNA damage accumulation was reported,⁵⁸ well in accordance with frequent *TP53* co-mutation. However, despite the loss of *p53*, our *H3G34R_ATRX* models exhibit clear signs of enhanced DNA damage stress compared to telomerase-driven pHGG subtypes. The *H3G34R* mutation was reported to be responsible for the reduced DNA repair capacity of a genetically modified DHG-H3G34R mouse model with *ATRX* and *TP53* loss.¹⁵ Accordingly, our iPSC data reveal enhanced DNA damage induced by *H3G34R_ATRX*, while no alterations were found after a single *H3G34R* mutation. Together, these observations suggest a cooperative role of both genomic alterations in the survival of cells under enhanced DNA damage responses. Supporting the in vitro data, clinical DHG-H3G34R_ATRX specimens showed increased DNA damage stress.

Consequently, we evaluated, if the enhanced DNA damage might represent an Achilles heel for improved therapy of DHG-H3G34R_ATRX. As PARP family proteins are crucial for maintaining genome integrity and have a central role in SSB and DSB repair, we aimed to specifically target DHG-H3G34R_ATRX with PARPi.^{12,13} Supporting this therapeutic strategy, PARP1 becomes activated in *ATRX*-mutated cells to protect stalled replication forks from degradation,²⁶ and BRCA1 is a top regulator of the cycling DHG-G34 tumor cell population.⁵⁹ Screening a pHGG patient-derived cell line panel, we observed insensitivity towards single agent PARPi. However, combinations with either topoisomerase or ATR/CHK1 inhibitors showed enhanced activity specifically in DHG-H3G34R_ATRX. Indeed, a synergistic effect of niraparib combined with topotecan was also induced by *ATRX*-KO in *H3G34R*-OE iPSCs, despite their telomerase-positive background. This was also reflected in CAM and PDX experiments. High variability and instability in tumor-take and progression concerning both in vivo models reflected general difficulties in modeling DHG-H3G34R_ATRX in a preclinical setting. Nevertheless, we observed in both models a significant reduction in tumor cell density. In the PDX model, loss of stemness characteristics was accompanied by induction of mature interneuronal markers, indicative of loss of DHG-H3G34R aggressiveness.⁵⁹

Regarding the current standard of care, some DHG-H3G34R tumors have been shown to harbor O⁶-methylguanine DNA methyltransferase (MGMT) promoter methylation,⁶⁰ suggesting TMZ sensitivity. Furthermore, DHG-H3G34R tumors have been described as responsive



Targeting DHG-H3G34R with PARPi and topoisomerase-I inhibitors is applicable in the clinical setting. MRI and ^{18} F-ethyl tyrosine positron emission (FET-PET) displaying the course of disease and therapy of a pediatric patient diagnosed with a DHG-H3G34R (corresponding to the VBT347 cell model). (A) Axial FLAIR image and contrast-enhanced (CE) T1-weighted MR image prior to primary surgery and postoperative axial CE T1-weighted MR image. (B) Axial FLAIR image and CE T1-weighted MR image at disease progression following irradiation (RTX) with concomitant temozolomide (TMZ) 3 months after initial diagnosis. The third image shows the color map of the choline/NAA ratios of a multivoxel MR spectroscopy (CSI; TE = 135 ms) at the level of one of the enhancing components. The colors red and green represent the voxels with high ratios. (C) Axial FLAIR image and CE T1-weighted MR image following 8 months of niraparib/topotecan treatment. In the third image (FLAIR) no signal changes are seen in the location of the subsequent recurrence. (D) Axial FLAIR image and CE T1-weighted MR image showing disease progression 13 months after initiation of niraparib/topotecan treatment, with a non-enhancing tumor manifestation rostral to the initial tumor sight with increased amino acid tracer uptake in an axial FET-PET/MR-fusion color map. The timeline starts with the presentation of the patient to the clinic (time point zero) and indicates further therapeutic interventions and therapy duration. The red arrows indicate tumor manifestation, and the green arrows point to areas of regressing tumor components.

to irradiation in a preclinical setting.¹⁵ The patient presented in this study, diagnosed as MGMT promoter-methylated, *TP53*-mutated DHG-H3G34R-*ATRX*, progressed immediately after irradiation, with an in-field recurrence. Based on our promising preclinical data, we decided to treat the patient (corresponding to VBT347) with off-label niraparib and topotecan combination. This decision was supported by 2 studies indicating the potential of implementing PARPis in

therapeutic regimens for pHGG; however, without considering the impact of DHG-H3G34R-*ATRX* background.^{61,62} In our DHG-H3G34R-*ATRX* case, the niraparib/topotecan regimen resulted in a partial response lasting for thirteen months. Importantly, this implies that niraparib can cross the blood-brain barrier at least in the malignant glioma tissue, in accordance with data published for brain metastases of endometrial and ovarian cancers.^{63,64} Considering

the relapse, other PARPi with a better PARP trapping potency, not available in time for the described case, might even exert enhanced responses in this combination setting. For sure, molecular mechanisms underlying resistance development and relapse need to be further investigated. Nevertheless, this responsive case provides the first preliminary evidence for the successful clinical use of PARPi combinations with DNA-damaging agents at least for standard therapy-resistant DHG-H3G34R_ATRX. Thus, clinical trials further investigating this combination strategy specifically in DHG-H3G34R_ATRX are warranted.

Supplementary material

Supplementary material is available online at *Neuro-Oncology* (<https://academic.oup.com/neuro-oncology>).

Keywords

ATRX | DNA damage | diffuse hemispheric glioma | H3G34R | PARP inhibitor

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Authorship statement

Conceptualization and study design: A.L., W.B., and J.G.; methodology: A.L., C.L., T.B., S.S.K., N.S.C., K.H., D.L.G., A.B., J.S., J.A.K., W.B., and J.G.; formal analysis and investigation: A.L., C.L., L.M., K.B., L.G., D.S., P.M., T.B., C.P., C.N.J., D.K., P.V., M.G., A.F., S.M., N.S.C., K.H., A.B., D.L.G., J.A.K., W.B., and J.G.; sequencing data analysis: LG (RNAseq), WMS (WES); radiological validation: M.T.S., T.T.W., P.K., and F.E.; neuropathological description: J.A.H. and C.H.; supporting material: N.S., B.R., A.B., K.D., C.D., A.P., A.A.A., and S.M.P.; writing—original draft preparation, A.L., C.L., L.M., W.B., and J.G. (with input from all coauthors); supervision: J.A.K., W.B., J.G.; funding acquisition: L.M., S.M., D.L.G., J.A.K., and J.G.; resources: S.S.K., K.H., J.S., J.A.K., W.B., and J.G..

Competing interests

A.L. is consultant and advisory board member for CCIE (not relevant to this study). A.A. is consultant and advisory board participant for Novartis (not relevant to this study). F.E. is advisory board participant for Servier and received honoraria from Dr. Sennewald Medizintechnik (not relevant to this study). S.M.P. is consultant for Epignostix GbmH and BioSkrbyb (not relevant to this study). W.B. received honoraria from BMS, Novartis, and Roche (not relevant to this study). J.G. is consultant, advisory board participant and received honoraria for Novartis and Roche (not relevant to this study).

Data availability

WES data of pHGG patient-derived cell models will be available upon request to the corresponding authors. Upon publication data will be deposited on the R2: Genomics Analysis and Visualization Platform (<http://r2.amc.nl>). Bulk RNA seq data of iPSCs will be available upon request to the corresponding authors.

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