Therapeutic targeting of PGBD5-induced DNA repair dependency in pediatric solid tumors

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Despite intense efforts, the cure rates of childhood and adult solid tumors are not satisfactory. Resistance to intensive chemotherapy is common, and targets for molecular therapies are largely undefined. We have found that the majority of childhood solid tumors, including rhabdoid tumors, neuroblastoma, medulloblastoma, and Ewing sarcoma, express an active DNA transposase, PGBD5, that can promote site-specific genomic rearrangements in human cells. Using functional genetic approaches, we discovered that mouse and human cells deficient in nonhomologous end joining (NHEJ) DNA repair cannot tolerate the expression of PGBD5. In a chemical screen of DNA damage signaling inhibitors, we identified AZD6738 as a specific sensitizer of PGBD5-dependent DNA damage and apoptosis. We found that expression of PGBD5, but not its nuclease activity–deficient mutant, was sufficient to induce sensitivity to AZD6738. Depletion of endogenous PGBD5 conferred resistance to AZD6738 in human tumor cells. PGBD5-expressing tumor cells accumulated unrepaired DNA damage in response to AZD6738 treatment and underwent apoptosis in both dividing and G1-phase cells in the absence of immediate DNA replication stress. Accordingly, AZD6738 exhibited nanomolar potency against most neuroblastoma, medulloblastoma, Ewing sarcoma, and rhabdoid tumor cells tested while sparing nontransformed human and mouse embryonic fibroblasts in vitro. Finally, treatment with AZD6738 induced apoptosis and regression of human neuroblastoma and medulloblastoma tumors engrafted in immunodeficient mice in vivo. This effect was potentiated by combined treatment with cisplatin, including substantial antitumor activity against patient-derived primary neuroblastoma xenografts. These findings delineate a therapeutically actionable synthetic dependency induced in PGBD5-expressing solid tumors.

INTRODUCTION

Despite improvements in intensive combination chemotherapy, surgery, and radiotherapy, treatment of the majority of childhood and adult solid tumors remains inadequate. For example, neuroblastomas and medulloblastomas characterized by amplifications of the MYCN and MYC oncogenes, respectively, remain mostly fatal (1–3). Likewise, cancers defined by mutations of the genes encoding the SWI/SNF chromatin remodeling complex, such as rhabdoid tumors, are almost uniformly incurable (4). Finally, the majority of human sarcomas, such as Ewing sarcoma, tend to be chemotherapy-resistant and lethal if they cannot be completely removed by surgery (5). The majority of refractory childhood solid tumors are characterized by mutations of factors that regulate gene expression or complex genomic rearrangements, both of which are not generally amenable to current pharmacologic strategies. Thus, new therapeutic approaches are urgently needed to improve the cure rates for these patients.

To enhance therapeutic efficacy, synthetic lethal cellular dependencies have been leveraged for cancer therapy (6). For example, tumors with inefficient homologous recombination DNA repair due to mutations of BRCA1/2 exhibit synthetic lethality with inhibitors of poly(adenosine diphosphate–ribose) polymerases, enabling substantial improvements in the treatment of patients (7, 8). In addition, synthetic dependencies in metabolic function (9), chromatin remodeling (10), and DNA damage signaling (11–13) are beginning to be explored to develop improved targeted therapies. In particular, intrinsic DNA damage due to replication stress or expression of oncogenes, such as MYC (14), and deficiencies in the DNA damage response due to mutations of TP53, ATM, or ATR confer susceptibility to specific inhibitors of DNA damage repair signaling (15). However, these mutations are generally rare in childhood cancers, and little is known about therapeutically targetable synthetic dependencies in pediatric solid tumors.

Recently, the human piggyBac transposable element derived 5 (PGBD5) was identified as an active DNA transposase that can mobilize synthetic DNA transposons in human cells (16). PGBD5-mediated DNA transposition requires the putative DDD catalytic triad in the PGBD5 transposase domain and specific DNA recognition sequences and target sites (16). PGBD5 is expressed in the majority of childhood solid tumors, including refractory rhabdoid tumors, where it promotes site-specific genomic rearrangements and mutations of tumor suppressor genes, at least in part due to the aberrant targeting of its DNA nuclease activity (17). This tumorigenic nuclease activity of PGBD5 raises the possibility that PGBD5–expressing cells may depend on active DNA damage repair and signaling.

Here, we report that PGBD5 activity confers a functional dependence on the KU complexes (XRCC5/XRCC6 and KU70/KU80) that binds DNA double-strand breaks (DSBs) and the ATR [ataxia telangiectasia mutated
(ATM) and Rad3 related] and ATM kinases that control DNA damage repair signaling in cells. We found that PGBDS activity is sufficient to confer this synthetic dependence, and endogenous PGBD5 expression is necessary to render childhood solid tumor cells susceptible to inhibitors of DSB repair signaling. As a result, pharmacologic targeting of DNA damage repair signaling using selective inhibitors exhibits therapeutic activity in multiple preclinical models of neuroblastoma, medulloblastoma, Ewing sarcoma, and rhabdoid tumors that express PGBDS in vitro and in vivo. The availability of clinical-grade inhibitors of DNA damage repair signaling offers immediate potential for translation into clinical trials for patients with refractory childhood solid tumors, the majority of which express PGBD5, as well as distinct subsets of PGBD5-expressing adult solid tumors.

RESULTS

PGBD5-expressing cells do not tolerate deficiency of nonhomologous end-joining DNA repair

Eukaryotic DNA transposases rely on cellular DNA repair mechanisms to restore intact target sites upon DNA rearrangements (18). In mammalian cells, this activity is principally carried out by the classic nonhomologous end joining (NHEJ) DSB repair apparatus (19). NHEJ repair consists of the heterodimeric Ku70/Ku80 complex that binds DSB ends and the DNA damage repair signaling factors including the ATM and ATR kinases that, in concert, lead to chromatin reorganization and DNA ligase–mediated DSB repair (20). Depending on the specific molecular features of DNA damage and cell state, assembly of different repair complexes can lead to activation of specific signaling pathways (21–23). This suggests that distinct forms of intrinsic DNA damage in cancer cells can be used selectively for their synthetic lethal targeting.

To test the cellular DNA repair requirements of PGBD5, we used mouse embryonic fibroblasts (MEFs) from mice deficient for Ku80−/−, Atm−/−, and the hypomorphic Seckel syndrome allele of AtrS/S and immortalized with the SV40 large T antigen (Fig. 1A) (24–26). Similar experiments using Lig4−/− MEFs could not be performed because of their severe proliferation defect (fig S1) (27). We used a doxycycline-inducible transgene encoding human PGBD5 and confirmed equal PGBD5 protein expression upon doxycycline induction using Western immunoblotting (Fig. 1A). Wild-type SV40 large T antigen–immortalized MEFs exhibited no measurable changes in cell growth upon doxycycline-induced PGBD5 expression (Fig. 1, B to E). In contrast, Atm−/−, AtrS/S and Ku80−/− MEFs underwent cell death, as detected by the significant accumulation of cleaved caspase-3 (ClCas3; P = 1.0 × 10−2, 8.0 × 10−3, and 1.0 × 10−3, respectively; Fig. 1, B and C), terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL; P = 1.0 × 10−2 and 2.0 × 10−3, respectively; Fig. 1D), and histone H2AX S139 phosphorylation (γH2AX; P = 3.0 × 10−2 and 2.0 × 10−3, respectively; Fig. 1E). Deficiency of Ku80, which functions in direct DSB binding during NHEJ DNA repair, exhibited similar levels of cell death as the deficiencies of Atr and Atr that contribute to the activation and propagation of DNA damage signaling, respectively (Fig. 1B). Thus, PGBD5

Fig. 1. PGBD5-expressing cells do not tolerate deficiency of nonhomologous end-joining DNA repair. (A) Western blot of PGBD5 protein expression after induction with doxycycline (DOX; 500 ng/ml for 24 hours) of SV40 large T antigen–immortalized MEFs deficient for Atm−/−, AtrS/S, or Ku80−/−. Actin serves as loading control. (B) Induction of apoptosis as measured by ClCas3 staining of MEFs deficient in DNA repair factors upon doxycycline-induced (48 hours) expression of PGBD5 (red) as compared to control phosphate-buffered saline (PBS)–treated cells (blue). *P = 0.010, 0.0080, and 0.0010 for Atm−/−, AtrS/S, and Ku80−/− of doxycycline versus control, respectively. (C) Representative photomicrograhs of MEFs upon doxycycline-induced PGBD5 expression for 48 hours (+) as compared to PBS-treated controls (−), stained for 4′,6-diamidino-2-phenylindole (DAPI; blue) and ClCas3 (red). (D) to (E) Induction of DNA DSBs measured by TUNEL staining (D) and γH2AX staining (E) of MEFs deficient in DNA repair factors upon doxycycline-induced (48 hours) expression of PGBD5 (red) compared to control PBS-treated cells (blue). *P = 0.0010 and 0.0020 for Atm−/− and AtrS/S of doxycycline versus control, respectively (D). *P = 0.0030 and 0.020 for Atm−/− and AtrS/S of doxycycline versus control, respectively (E). Error bars represent SDs of three biological replicates.
expression requires the cellular NHEJ and DNA damage signaling apparatus.

**PGBD5 expression is sufficient to confer susceptibility to inhibition of DNA damage repair signaling**

On the basis of the finding that cells expressing PDBD5 are dependent on active NHEJ DNA damage repair and signaling, we hypothesized that PGBD5 expression might render cells susceptible to pharmacological inhibition of DNA damage signaling. To test this idea, we used primary human retinal pigment epithelial (RPE) cells immortalized with telomerase that allow the investigation of DNA damage response in genomically stable, nontransformed human cells (28). We reasoned that specific PGBD5-dependent DNA damage response requirements may be identified by comparative analysis of growth and survival of RPE cells expressing GFP-PGBD5, as compared to GFP control and its catalytically inactive GFP-PGBD5 D168A/D194A/D386A mutant. This mutant is expressed equally to wild-type PGBD5, as assessed by Western immunoblotting, and physically associates with chromatin, as assessed by chromatin immunoprecipitation and DNA sequencing, but does not support DNA transposition in reporter assays (17). To minimize the possible contribution of secondary effects of PGBD5 expression due to its induction of mutagenic DNA rearrangements and cell transformation, we used cells for experiments immediately after transgene transduction (17).

Thus, we screened a panel of commercially available inhibitors of DNA damage signaling for their ability to selectively interfere with the growth and survival of RPE cells expressing GFP-PGBD5, as compared to wild-type or control transgene-expressing cells (Fig. 2A, Fig. 2S, and table S1). In particular, we observed that the ATR- and ATM-selective kinase inhibitors, AZD6738 and KU60019, exhibited more than 20- and 5-fold enhanced activity, respectively, against RPE cells expressing GFP-PGBD5, as compared to GFP control (Fig. 2A). Consistent with the notion that tolerance of PGBD5 DNA nuclease activity requires active DNA repair in cells, mutation of the putative aspartate catalytic triad (D168A, D194A, and D386A), thought to catalyze phosphodiester bond hydrolysis during transposase-induced DNA rearrangements (16), completely abrogated the enhanced susceptibility of RPE cells upon PGBD5 expression (Fig. 2B). We confirmed that AZD6738 selectively inhibits ATR as compared to ATM (29, 30), insofar as itActive ATP- and ATR-P20 MEFs exhibited, respectively, increased and diminished susceptibilities to AZD6738, in agreement with its reported selectivity profile (IC50 = 0.023 and 2.0 μM, respectively, as compared to 0.36 μM for wild-type control; Fig. 2C) (11–13). Thus, PGBD5-dependent effects may be explained by the selective inhibition of DNA damage signaling by ATR- and ATM-selective AZD6738 and KU60019, respectively. Lack of PGBD5-dependent effects of other potent DNA damage signaling inhibitors is presumably related to their respective selectivity profiles, such as AZ20, which potently inhibits ATM, ATR, and mTOR, and VE822 (VX-970), which potently inhibits both ATR and ATM kinases (table S1).

As with the functional genetic requirements of DNA repair and DNA damage signaling induced by PGBD5 expression in MEFs (Fig. 1), we found that active DNA damage signaling blocked by AZD6738 is also required for the growth and survival of PGBD5-expressing human RPE cells (Fig. 2, D to H). Specifically, we found that RPE cells expressing GFP-PGBD5, but not those expressing GFP or the inactive GFP-PGBD5 mutant, exhibited significantly increased DNA damage upon treatment with AZD6738, as measured by γH2AX staining analysis (P = 0.040; Fig. 2, D and G). Similarly, we observed significantly increased TUNEL levels in RPE cells expressing GFP-PGBD5 upon treatment with AZD6738, whereas cells expressing control GFP or the catalytically inactive GFP-PGBD5 mutant exhibited unperturbed steady-state background TUNEL levels (P = 5.6 × 10−6; Fig. 2, E and H). Consistent with the notion that wild-type PGBD5 is actively inducing DSBs in cells, we observed significantly increased TUNEL levels even in the absence of AZD6738 treatment, an effect that was completely abolished by the mutation of its putative nuclease catalytic triad (Fig. 2, E and H). TUNEL accumulation upon PGBD5 expression and AZD6738 treatment was predominantly observed in the G1 phase of the cell cycle (Fig. 2E). In accordance with the accumulation of unrepaired DNA damage upon AZD6738 treatment in PGBD5-expressing cells, we observed significantly increased apoptosis, as measured by caspase-3 cleavage, in cells expressing GFP-PGBD5 compared to those expressing its inactive mutant or GFP control (P = 3.0 × 10−2; Fig. 2F). Consistent with the lack of apparent effect of AZD6738 on TUNEL in S-phase cells (Fig. 2E), we confirmed that the PGBD5-specific susceptibility to AZD6738 was not immediately associated with DNA replication stress, as assessed by Western immunoblotting of phosphorylated replication protein A 32 kDa (RPA32) T21 and S4/8, compared to the DNA topoisomerase I inhibitor camptothecin, which predominantly induces DSBs during DNA replication in the S phase (Fig. 3) (31). In agreement with this, RPE cells expressing GFP-PGBD5 did not show increased cell cycling compared to cells expressing GFP control, as measured by 5-ethyl-2'-deoxyuridine incorporation (fig. S3). Last, in agreement with site-specific accumulation of DSBs (32), we observed mostly punctate, as opposed to pan-nuclear, γH2AX accumulation in PGBD5-expressing cells treated with AZD6738 (fig. S4). Thus, PGBD5 expression is sufficient to confer specific susceptibility to pharmacologic inhibitors of DNA damage signaling, such as AZD6738.

**Rhabdoid tumor, medulloblastoma, neuroblastoma, and Ewing sarcoma cells that express PGBD5 exhibit enhanced sensitivity to AZD6738**

Previously, we observed that PGBD5 is expressed in the majority of childhood solid tumors, including neuroblastoma, medulloblastoma, Ewing sarcoma, and rhabdoid tumors (17). In particular, in rhabdoid tumors, we found that the DNA recombinase activity of PGBD5 was necessary and sufficient to induce genomic rearrangements in both rhabdoid tumor cell lines and patient primary tumors. We have now found that expression of PGBD5 in nontransformed MEFs and human RPE cells is sufficient to induce DNA damage, as measured by TUNEL incorporation in cells, which can be potentiated by the DNA damage signaling inhibitor AZD6738. Thus, we reasoned that AZD6738 may exhibit antitumor activity against childhood solid tumor cells expressing PGBD5.

To test this idea, we treated a panel of rhabdoid tumor, neuroblastoma, medulloblastoma, and Ewing sarcoma cell lines, as well as nontransformed human RPE and BJ (human foreskin fibroblasts immortalized with human telomerase reverse transcriptase) cells with AZD6738. We observed that the 19 childhood tumor cell lines tested exhibited enhanced sensitivity to AZD6738, with IC50 values largely in the nanomolar range (Fig. 4A, fig. S5, and table S2). In contrast, nontransformed RPE and BJ cells were relatively resistant to AZD6738, with IC50 values in the high micromolar range (Fig. 4A and fig. S5). The susceptibility to AZD6738, as measured by its IC50 values in vitro, exhibited a significant correlation with the expression of PGBD5 protein, assessed by quantitative fluorescent Western immunoblotting (P = 4.4 × 10−3; Fig. 4B and figs. S5 and S6). This association did not appear to segregate with tumor tissue type (Fig. 4B) or the presence of mutations in genes...
known to affect DNA damage signaling, such as TP53, ATM, ATR, MYC, MYCN, XRCC3, XRCC5, CHK1, BRCA2, RAS, and ATRX (fig. S6 and table S2). In addition, because ATM deficiency can confer increased sensitivity to AZD6738 (12), we confirmed that PGBD5 expression did not affect the expression of ATM itself in RPE cells and that human tumor cells exhibiting enhanced susceptibility to AZD6738 lacked ATM mutations and retained ATM protein expression (fig. S7 and table S2).

In agreement with observations of MEFs and RPE cells expressing PGBD5, human tumor cell lines expressing endogenous PGBD5 and treated with AZD6738 underwent apoptosis and accumulated unrepaired...
DNA damage, as measured by caspase-3 cleavage and TUNEL staining (Fig. 4, C to F). Most of the TUNEL incorporation induced by AZD6738 in PGBD5-expressing tumor cells was observed in G1-phase cells (Fig. 4E and fig. S8). Thus, AZD6738 exhibits antitumor efficacy against PGBD5-expressing childhood solid tumors in vitro.

Endogenous PGBD5 is necessary to confer enhanced susceptibility to inhibitors of DNA damage signaling in tumor cells

Because ectopically expressed PGBD5 may induce DNA damage and signaling dependencies not present in human tumors with endogenous PGBD5 expression, we sought to determine whether endogenous PGBD5 expression is required for the enhanced susceptibility to inhibitors of DNA damage signaling. To achieve this, we identified two independent lentiviral RNA interference vectors expressing short hairpin RNA (shRNA) against human PGBD5 that substantially depleted endogenous PGBD5 at both the mRNA and protein levels, as compared to control vectors targeting GFP (Fig. 5A) (17). The degree of PGBD5 depletion appeared to depend on tumor type, with rhabdoid G401 cells exhibiting a more than 15-fold reduction in mean PGBD5 expression, whereas neuroblastoma IMR5 and medulloblastoma HD-MB03 cells exhibited reduced effects (Fig. 5A). Notably, we observed that depletion of PGBD5 reduced the viability of neuroblastoma cells (fig. S9), whereas PGBD5 was dispensable for survival of rhabdoid tumor cells (17).

Consistently, we found that depletion of PGBD5 induced relative resistance to AZD6738 compared to wild-type cells or cells transduced with control vector shRNA-targeting GFP (shGFP), as evidenced by the relative increase in the IC_{50} values for rhabdoid tumor G401 and medulloblastoma HD-MB03 cells (P < 1 × 10^{-4} and P = 0.0056 for

Fig. 3. Treatment with AZD6738 induces PGBD5-dependent DNA damage.

Western immunoblot of RPE cells expressing GFP-PGBD5 or GFP and treated with 500 nM AZD6738 for 6 hours, as indicated. PGBD5 expression increases the amounts of γH2AX and TP53 pS15, but not RPA32 pT21 or pS4/8, upon combination with AZD6738 treatment. Actin serves as loading control, and treatment with 1.5 μM DMSO for 2 hours serves as positive control for replication stress-mediated induction of RPA32 phosphorylation. Arrowhead marks the specific RPA32 pT21 band.

Fig. 4. PGBD5-expressing rhabdoid tumor, medulloblastoma, neuroblastoma, and Ewing sarcoma cells exhibit enhanced susceptibility to AZD6738. (A) Susceptibility to AZD6738, shown by its IC_{50} values upon 120 hours of treatment of wild-type MEFs and BJ fibroblasts (normal), rhabdoid tumor (RT), medulloblastoma (MB), neuroblastoma (NB), and Ewing sarcoma (ES) cell lines. Complete list of cell lines and their susceptibility to AZD6738 is shown in table S2 and fig. S5. (B) PGBD5 protein expression as measured by Western immunoblotting (fig. S6) is significantly associated with the susceptibility to AZD6738 in pediatric tumor cell lines. Lines denote the 95% confidence interval of linear regression (P = 0.0044). Points are labeled according to the cell line scheme in (A). (C) Induction of apoptosis, as measured by caspase-3 cleavage of IMR5 neuroblastoma, G401 rhabdoid tumor, HD-MB03 medulloblastoma, and TC71 Ewing sarcoma cells with 500 nM AZD6738 for 72 hours (red) compared to DMSO control (blue). *P = 6.1 × 10^{-4} and 3.9 × 10^{-4} for AZD6738 versus DMSO of IMR5 and HD-MB03, respectively. (D) Representative photomicrographs of IMR5 neuroblastoma cells after treatment with 500 nM AZD6738 (+) or DMSO control (−) for 72 hours, stained for Cleaved caspase 3 (red) and DAPI (blue). (E) Representative flow cytometry profile of TUNEL and propidium iodide incorporation into HD-MB03 cells after treatment with 500 nM AZD6738 or DMSO control for 48 hours. (F) Induction of TUNEL upon treatment with 500 nM AZD6738 (red) versus DMSO control (blue) for 48 hours. *P = 0.042, 0.025, 5.2 × 10^{-5}, and 2.0 × 10^{-5} for IMR5, G401, HD-MB03, and TC71, respectively. Error bars represent SDs of three biological replicates.
Untransduced
shGFP
shPGBD5-1
shPGBD5-2

IMR5
G401
HD-MB03

shPGBD5-1
shGFP
shPGBD5-2

shPGBD5-1
shPGBD5-2

shPGBD5-1
shGFP
shPGBD5-2

AZD6738 (μM)

Fig. 5. PGBD5 expression is necessary for tumor cell susceptibility to AZD6738. (A) PGBD5 mRNA expression in cells transduced with shRNA against PGBD5, as compared to shGFP and untransduced control cells. (B to D) Dose response of childhood tumor cell lines IMR5 (B), G401 (C), and HD-MB03 (D) treated with varying concentrations of AZD6738 for 120 hours upon PGBD5 depletion. Error bars represent SDs of three replicates.

shPGBD5-1 and shPGBD5-2 in G401 cells, respectively, and \( P = 4.0 \times 10^{-3} \) and \( P = 0.068 \) for shPGBD5-1 and shPGBD5-2 in HD-MB03 cells, respectively; Fig. 5, B to D, and table S3), whereas this effect was not as pronounced in neuroblastoma IMR5 cells (Fig. 5B), presumably due to the PGBD5-dependent effects on cell survival (fig. S9). Likewise, depletion of endogenous PGBD5 also conferred relative resistance to the ATM-selective inhibitor KU60019, supporting the notion that PGBD5 induces intrinsic DNA damage and requires ongoing DNA damage signaling in tumor cells (fig. S10). Thus, PGBD5 expression is necessary to confer susceptibility to inhibitors of DNA damage signaling, such as AZD6738, in diverse childhood solid tumors.

**AZD6738 induces DNA damage and apoptosis and exhibits antitumor efficacy in xenograft models of high-risk human neuroblastoma and medulloblastoma in vivo**

Compelled by the findings that AZD6738 induced PGBD5-dependent DNA damage and apoptosis in pediatric solid tumor cell lines in vitro, we set out to test whether single-agent AZD6738 treatment has antitumor activity in preclinical models of pediatric solid tumors in vivo. First, we chose to investigate its activity against mouse xenografts of high-risk MYCN-amplified neuroblastoma (IMR5), high-risk MYC group 3 medulloblastoma (HD-MB03), refractory rhabdoid tumor (G401), and Ewing sarcoma (TC71) cells because they represent the most common refractory childhood solid tumors (33, 34). Thus, we transplanted IMR5, HD-MB03, G401, and TC71 cells subcutaneously in athymic nude Foxn1nu immunodeficient mice and monitored tumor growth upon oral treatment of mice with AZD6738 (50 mg/kg per day) compared to vehicle control (Fig. 6 and fig. S11). We found that AZD6738 treatment significantly impaired the growth of both neuroblastoma IMR5 and medulloblastoma HD-MB03 tumors, as compared to vehicle control–treated mice (\( P = 4.9 \times 10^{-3} \) and \( 5.5 \times 10^{-6} \) at day 28, respectively; Fig. 6, A and B). The magnitude of this effect appeared substantial as compared to the previously reported effects of AZD6738 against tumors with genetic deficiencies of ATM, XRCC1, or ERCC1 (35–38). Similar effects were observed using Kaplan-Meier survival analysis (log-rank \( P = 1.0 \times 10^{-2} \) and \( 1.0 \times 10^{-4} \), respectively; fig. S11). There was no evident toxicity of this treatment, as assessed by the unchanged animal body weights (fig. S12). AZD6738 did not have single-agent antitumor activity against G401 and TC71 cells in vivo, suggesting that tumor-specific differences in DNA repair and DNA damage signaling may affect therapeutic targeting of PGBD5-induced DNA repair dependencies (fig. S11). Residual tumor cells isolated from mice upon the completion of 20 days of AZD6738 treatment exhibited significantly reduced proliferation, as measured by Ki67 staining (\( P = 3.1 \times 10^{-5} \) and \( 1.0 \times 10^{-3} \) for IMR5 and HD-MB03, respectively; Fig. 6, C and D), and increased DNA damage and apoptosis, as measured by γH2AX and ClCas3 staining, respectively (\( P = 1.4 \times 10^{-3} \) and \( 4.3 \times 10^{-4} \) for γH2AX in IMR5 and HD-MB03, respectively; Fig. 6, C, E, and F). In addition, we also assessed the activity of VE822, an ATR/ATM inhibitor that is currently in clinical trials (39), against neuroblastoma IMR5 and rhabdoid G401 cell line xenografts. In contrast to the ATR-selective AZD6738, single-agent treatment with VE822 had no significant effects on tumor growth in vivo (fig. S13), supporting the notion that pharmacologic and selectivity properties of inhibitors of DNA damage signaling may also affect the efficacy of PGBD5-dependent therapeutic targeting.

**Synergistic therapy targets PGBD5-induced DNA repair dependency in patient-derived primary neuroblastoma xenografts in vivo**

Considering that AZD6738 exhibited potent single-agent activity against high-risk neuroblastoma and medulloblastoma cell line xenografts, but not against rhabdoid tumor and Ewing sarcoma xenografts, we reasoned that specific agents that induce PGBD5-dependent DNA damage repair and/or signaling may selectively potentiate the antitumor effects of AZD6738 against tumors with genetically low combination indices for the neuroblastoma, medulloblastoma, rhabdoid tumor, and Ewing sarcoma cell lines (Fig. 7A) (42).

To investigate the potential therapeutic benefit of combining AZD6738 with cisplatin therapy as a prelude to its clinical testing in patients, we established two patient-derived primary xenografts, PDX1 and PDX2, from non–MYCN-amplified, stage IV metastatic neuroblastoma tumors obtained at diagnosis before initiation of clinical therapy (Fig. 7B and table S4). In agreement with the results in human...
tumor cell lines (Fig. 4), we observed different expression of PGBD5 in the two neuroblastoma xenografts (Fig. 7B). Subsequently, we transplanted these tumor specimens subcutaneously into NOD.Cg-Prkdc<sup>−/−</sup>Il2rg<sup>−/−</sup>Sug/jicTac (NOG) immunodeficient mice and, upon tumor engraftment as assessed by tumor volumetric measurements, randomized recipient mice to be treated with single-agent AZD6738 (50 mg/kg, per os, daily for 14 days), single-agent cisplatin (2 mg/kg, intraperitoneally (ip) every 7 days), combination of AZD6738 and cisplatin, or vehicle control (Fig. 7, C and D). Consistent with the relatively low expression of PGBD5 in PDX1 neuroblastoma tumor (Fig. 7B), we found that single-agent treatment with AZD6738 or cisplatin had no effect, whereas combination of AZD6738 with cisplatin exhibited significant reduction in tumor growth, as compared to single-agent or vehicle control treatments (<i>P</i> = 1.9 × 10<sup>−5</sup>; Fig. 7C). Likewise, for PDX2 neuroblastoma tumor, which had relatively high PGBD5 expression (Fig. 7B), we observed significant reduction in tumor growth upon single-agent AZD6738 and cisplatin treatment, as compared to vehicle control (<i>P</i> = 0.032 and 0.079, respectively; Fig. 7D). Together, these results indicate that AZD6738 exhibits single-agent and cisplatin-combination efficacy against PGBD5-expressing solid tumors.

**DISCUSSION**

We have found that the PGBD5 DNA transposase expressed in the majority of childhood solid tumors confers a synthetic dependency on DNA damage repair and signaling. Consistent with the genomic rearrangements promoted by PGBD5 in rhabdoid tumors (17), expression of PGBD5 induces DNA damage, which requires both DNA damage repair and DNA damage signaling, resulting in apoptosis if impaired by their selective inhibitors (fig. S14). Both primary mouse and human cells engineered to express PGBD5, as well as PGBD5-expressing childhood solid tumors, accumulated unrepaired DNA damage and underwent apoptosis upon treatment with selective inhibitors of DNA damage signaling. This effect was due to the specific nuclease activity of PGBD5, insofar as mutation of its putative catalytic nuclease residues completely abrogated the dependence on DNA damage signaling. In turn, single-agent treatment with the DNA damage signaling inhibitor AZD6738 exhibited potent antitumor activity against high-risk neuroblastomas and medulloblastomas with high expression of PGBD5 in preclinical mouse models in vivo.

Human cancers harbor various mechanisms of endogenous DNA damage as a source of genetic mutations and requirements for active DNA damage repair (43). As a result, selective inhibitors of DNA damage
the G1 phase without measurable replication stress or ATM depletion, it is DNA damage and apoptosis induced by AZD6738 appeared to occur in components of the DNA damage response. Unrecognized DNA or chromatin structures that recruit specific components of DNA damage signaling may be due to the PGBD5-induced generation of yet DNA bound by RPA. ATM are activated by specific DNA structures, including single-stranded DNA breaks (DSBs) of four individual xenografted mice per group. However, chemical DNA damage signaling kinase inhibitors exhibited antitumor activities in specific cancer types. In particular, selective inhibitors of ATR and ATM kinases have been used to target tumors with intrinsic deficiencies in DNA repair, as well as DNA damage susceptibility, such as that induced by oncogene and replication stress or alternative lengthening of telomeres (ALT) activities. Our current work revealed a specific synthetic dependency conferred by the endogenous DNA transposase PGBD5 in the majority of childhood solid tumors. Genetic experiments showed equivalent functional requirements for the scaffolding KU complex that directly binds DSBs and the ATR and ATM kinases that mediate DNA damage signaling. However, chemical DNA damage signaling kinase inhibitors exhibited a specific response profile, with the ATR- and ATM-selective inhibitors AZD6738 and KU60019 exhibiting enhanced PGBD5-dependent activity. Given their varied potency and selectivity, it is possible that other selective DNA damage signaling inhibitors can also effectively target PGBD5-induced tumor synthetic dependencies. Because ATR and ATM can have varying activity of NHEJ repair, as partly affected by neurotrophin receptor signaling, which may affect the therapeutic efficacy of targeting PGBD5-induced DNA repair dependencies. Such a model, in which intrinsic differences in DNA repair and DNA damage signaling affect therapeutic targeting of PGBD5-induced DNA repair dependencies, is supported by both the apparent variation in tumor responses to AZD6738 and its synergy with cisplatin therapy.

We observed that PGBD5 expression was required for the growth and survival of neuroblastoma cells, in contrast to rhabdoid tumor cells, where its expression is dispensable in vitro. Insofar as PGBD5 remains physically associated with its DNA rearrangements and repair, PGBD5 may regulate expression, as determined by the variation in tumorigenic mechanisms and phenotypic states of respective tumor cells of origin. For example, neuroblastoma cells can have varying activity of NHEJ repair and DNA damage signaling affecting therapeutic targeting of PGBD5-induced DNA repair dependencies.

Finally, our findings may explain the apparent activity of other kinase inhibitors, such as the observed antitumor activity of dactolisib (NVP-BEZ235) in neuroblastoma, medulloblastoma, and Ewing sarcoma (53–57), given its potent inhibition of the ATR kinase. Inhibitors of DNA damage signaling, including AZD6738, are currently being investigated in clinical trials (NCT02264678, NCT02223923, and NCT02630199). Our findings warrant their investigation in clinical trials for children with solid tumors, the majority of which express PGBD5 and should be susceptible to targeted therapy of this synthetic DNA damage signaling dependency. We anticipate that improved understanding of the molecular synthetic dependencies and their targeting by emerging selective pharmacologic inhibitors should provide rational therapeutic strategies for refractory solid tumors.

MATERIALS AND METHODS

Study design

The objective of our study was to identify specific vulnerabilities in PGBD5-expressing cancers. Using functional genetic and pharmacologic experiments, we found that PGBD5 expression renders cells susceptible to treatment with the ATR-selective inhibitor AZD7638. The
evaluation of cell survival, cell death, and DNA damage was performed using luminescence-based cell viability assays, fluorescence-activated cell sorting–based assays, as well as immunofluorescence and Western immunoblotting. We used 19 cell lines and four xenograft mouse models of pediatric solid tumors, as well as two patient-derived primary tumor specimens to analyze the effects of single-agent and combination therapy in vivo and in vitro. Tolerability of AZD6738 therapy was tested in mice by measuring body weights. In all experiments, animals were randomized to treatment groups without blinding. Sample sizes were estimated using statistical power analyses, as specified in the figure legends. All experiments were done in at least three biological replicates, if not otherwise specified in the manuscript text.

Statistical analysis
For comparisons between two sample sets, statistical analysis of means was performed using two-tailed, unpaired Student’s t tests. Survival analysis was done by the Kaplan-Meier method, as assessed using a log-rank test.

SUPPLEMENTARY MATERIALS
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Materials and Methods
Fig. S1. Ligase 4
Fig. S2. PGBD5 expression is sufficient to confer sensitivity to ATM and ATR inhibitors.
Fig. S3. GFP-PGBD5 expression in RPE cells does not affect cell cycle kinetics.
Fig. S4. AZD6732 induces punctate rH2AX accumulation.
Fig. S5. Rhodoid tumor, medulloblastoma, neuroblastoma, and Ewing sarcoma cells are sensitive to AZD6738.
Fig. S6. Expression of PGBDS in tumor cell lines correlates with their susceptibility to AZD6738, unlike TP53 and MYC/N status.
Fig. S7. ATM is expressed in PGBDS-expressing normal and tumor cell lines.
Fig. S8. AZD6738 treatment leads to increased DNA DSB as evidenced by TUNEL.
Fig. S9. Neuroblastoma cell lines do not tolerate PGBDS depletion.
Fig. S10. PGBDS expression is necessary for tumor cell susceptibility to KU60019.
Fig. S11. AZD6738 treatment decreases pediatric xenograft tumor growth in mice.
Fig. S12. Mouse weights are not affected by treatment with AZD6738.
Fig. S13. VE822 treatment does not inhibit pediatric xenograft tumor growth in mice.
Fig. S14. PGBDS induces a synthetic lethal DNA damage signaling dependency.
Table S1. Selectivity profiles of inhibitors of DNA damage signaling.
Table S2. Mutational profiles of genes that affect susceptibility of cells to inhibitors of DNA damage.
Table S3. Statistical analysis of drug susceptibility upon PGBD5 depletion.
Table S4. Demographic and molecular features of neuroblastoma patients.
Table S5. Oligonucleotide primers.
Table S6. Antibodies.
References (58-69)

REFERENCES AND NOTES
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Therapeutic targeting of PGBD5-induced DNA repair dependency in pediatric solid tumors


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Synthetic lethality, pediatric edition

Although a variety of therapeutic regimens are available for pediatric solid tumors, they are often ineffective and typically nonspecific. Henssen et al. determined that expression of a DNA transposase called PGBD5 is common in these tumors and presents a therapeutic vulnerability. The authors demonstrated that cells expressing PGBD5 are dependent on DNA repair through nonhomologous end joining, then identified a drug that inhibits this DNA repair pathway and is therefore active against many pediatric tumor types, particularly when combined with chemotherapy, while sparing surrounding nontumor tissues.