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PRECLINICAL STUDIES

ATP-binding cassette transporters limit the brain penetration of Wee1 inhibitors

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Summary *Introduction* Wee1 is an important kinase involved in the G2 cell cycle checkpoint and frequently upregulated in intracranial neoplasms such as glioblastoma (GBM) and diffuse intrinsic pontine glioma (DIPG). Two small molecules are available that target Wee1, AZD1775 and PD0166285, and clinical trials with AZD1775 have already been started. Since GBM and DIPG are highly invasive brain tumors, they are at least to some extent protected by the blood-brain barrier (BBB) and its ATP-binding cassette (ABC) efflux transporters. *Methods* We have here conducted a comprehensive set of *in vitro* and *in vivo* experiments to determine to what extent two dominant efflux transporters in the BBB, P-gp (ABCB1) and BCRP (ABCG2), exhibit affinity towards AZD1775 and PD0166285 and restrict their brain penetration. *Results* Using these studies, we demonstrate that AZD1775 is efficiently transported by both P-gp and BCRP, whereas PD0166285 is only a substrate of P-gp. Nonetheless, the brain penetration of both compounds was severely restricted *in vivo*, as indicated by a 5-fold (PD0166285) and 25-fold (AZD1775) lower brain-plasma ratio in wild type mice compared to Abcb1a/b;Abcg2$^{-/-}$ mice. *Conclusion* The brain penetration of these Wee1 inhibitors is severely limited by ABC transporters, which may compromise their clinical efficacy against intracranial neoplasms such as DIPG and GBM.

Keywords Glioma · Pharmacokinetics · Wee1 · ABC transporters · AZD1775 · PD0166285

Introduction

The eukaryotic cell cycle is tightly regulated and consists of four phases. Cells predominantly spend time in the G1/G0 phase and subsequently progress through S and G2 phase before undergoing mitosis. Each of these four cell cycle phases is equipped with a checkpoint. These checkpoints are intricate molecular switches that govern the decision to progress to the next phase in the cycle and are pivotal to maintaining genomic integrity in healthy cells [1]. Cancer cells however, frequently harbor a deregulated cell cycle. Most often, the G1 checkpoint is abrogated (e.g. due to loss of p53 function) and as a result cancer cells rely more heavily on the G2 checkpoint [2]. The G2 checkpoint is responsible for the decision to enter mitosis, and represents the final opportunity for the cell to minimize DNA damage before entering mitosis [3]. Inhibiting the G2 checkpoint in combination with DNA damage-inducing chemotherapy or radiotherapy has been postulated as a logical rationale to induce death of cancer cells harboring an abrogated G1 checkpoint. Forcing cells through division while sustaining considerable DNA damage creates high levels of genomic instability, ultimately resulting in mitotic catastrophe [4]. A promising target for this strategy is the kinase Wee1, a key enforcer of the G2 checkpoint.

Since the beginning of this century, two small-molecule Wee1 inhibitors have been developed (Fig. 1). PD0166285

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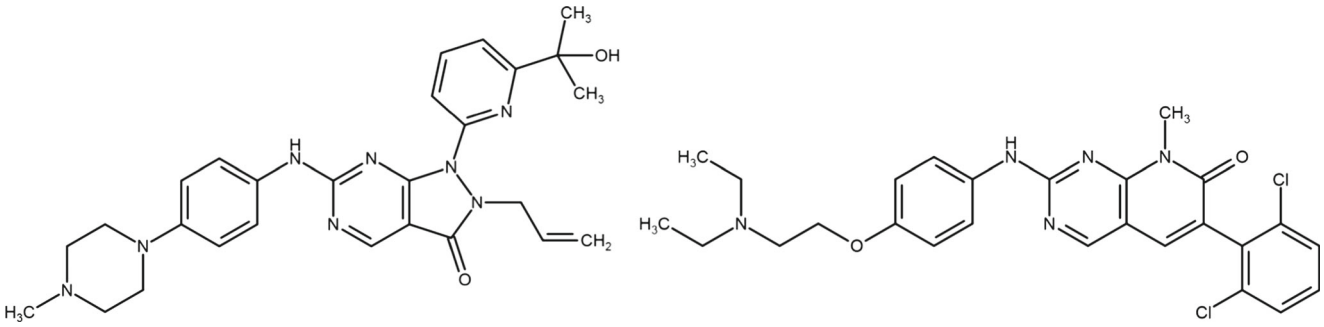

Fig. 1 Chemical structures of AZD1775 and PD0166285. Chemical structures of the Wee1 inhibitors AZD1775 and PD0166285. Both compounds contain the same core diphenylamine moiety

was discovered in 2001 as a Wee1 inhibitor that also targets MYT1 and CHK1 [5]. More recently, the specific Wee1 inhibitor AZD1775 (previously MK1775) was developed [6]. Both compounds have initially demonstrated preclinical efficacy in p53-mutated tumor models as sensitizers to both chemotherapy [7] and radiotherapy [5, 8]. Later, efficacy was also found in p53 wildtype tumors, and this effect was postulated to be mediated by intrinsic chromosomal instability [9] and replication stress [10, 11].

Inhibition of Wee1 has initially been investigated in extracranial tumor models only, and these preclinical studies have now led to clinical development of AZD1775. The results of the first phase II trial investigating AZD1775 as a chemosensitizer in p53-mutated ovarian cancer have now been reported, and appear very encouraging [12]. More recently however, Wee1 has been gaining attention for treatment of various intracranial tumors, such as glioblastoma (GBM) [13, 14], and diffuse intrinsic pontine glioma (DIPG) [15]. In these tumor types, Wee1 was shown to be one of the most overexpressed kinases and Wee1 inhibition could radiosensitize orthotopic preclinical GBM and DIPG models. These reports have now led to a number of clinical trials investigating AZD1775 in intracranial tumors such as GBM (Clinicaltrials.gov identifier NCT02207010, NCT01849146), DIPG (Clinicaltrials.gov identifier NCT01922076) and medulloblastoma, neuroblastoma and supratentorial embryonal tumors (Clinicaltrials.gov identifier NCT02095132).

When targeting intracranial tumors, at least one additional hurdle needs to be overcome, as these tumors are all to some extent protected by the blood-brain barrier (BBB) [16]. The brain microvascular endothelial cells form the core of the BBB and do not only prevent paracellular diffusion due to their lack of fenestrae and abundance of tight junctions, but are also equipped with a range of efflux transporters that actively pump out xenobiotics back into the bloodstream. These ATP-binding cassette (ABC) transporters, of which breast cancer resistance protein (BCRP; ABCG2) and P-glycoprotein (P-gp; ABCB1) are the most dominant, restrict the brain penetration of a wide range of xenobiotics, including many anticancer agents [17–19]. This impaired brain penetration has been demonstrated to limit therapeutic efficacy in several clinically relevant mouse models [20, 21]. Importantly, studies testing a panel of inhibitors targeting PI3K have shown that the most brain penetrable compounds were the most likely candidates to achieve intracranial antitumor efficacy [22, 23]. Since this might be similarly true for compounds targeting Wee1, we investigated the brain penetration of Wee1 inhibitors. We here show that both available Wee1 inhibitors, AZD1775 and PD0166285, are substrates of ABC transporters, potentially limiting their suitability for use against intracranial neoplasms such as GBM and DIPG.

Methods

Drugs

PD0166285 was purchased from Sigma-Aldrich (St Louis, MO) and AZD1775 (MK1775) from Axon Medchem BV (Groningen, The Netherlands). Zosuquidar was obtained from Eli Lilly (Indianapolis, IN). Elacridar was generously provided by GlaxoSmithKline (Research Triangle Park, NC).

Cell culture

All used cell lines were previously generated in-house by Dr. A.H. Schinkel [24–26]. Parental LLC-PK1 cells, and subclones overexpressing human ABCB1 (LLC-MDR1) or murine Abcb1a (LLC-Mdr1a), parental MDCK cells and sub-lines overexpressing human ABCG2 (MDCK-BCRP) or murine Abcg2 (MDCK-Bcrp1) were all cultured as described previously [18].

Concentration equilibrium transport assays

Concentration equilibrium transport assays (CETAs) were carried out using 500 nM of the Wee1 inhibitors and 5 μM of zosuquidar or elacridar was used to block transport, as described previously [18]. To prepare CETA samples for

subsequent HPLC analysis, medium samples were mixed with two volumes of acetonitrile. After centrifugation, the supernatant was diluted 3-fold with water and the concentration of AZD1775 or PD0166285 was measured by High Performance Liquid Chromatography (HPLC) coupled to a UV detector (Model 996 UV Photodiode Array detector; Waters, Milford, MA) using a GraceSmart RP18 5 μm column (150 × 2 mm) (Grace, Deerfield, IL). AZD1775 was detected at 340 nm using isocratic conditions with 45% acetonitrile in 0.1% (*v*/v) formic acid in water delivered at a flow rate of 0.2 mL/min. PD0166285 was detected at 360 nm using the same column eluted with a gradient of methanol and 0.1% (v/v) formic acid in water ranging from 30% to 70% delivered at a flow rate of 0.2 mL/min.

Animals

All animal studies were approved by the animal experiment committee and adhered to national law and institutional guidelines. The animals had access to food and water ad libitum.

Pharmacokinetic studies

We used wildtype, Abcg2$^{-/-}$, Abcb1a/b$^{-/-}$ and Abcg2;Abcb1a/b$^{-/-}$ FVB mice. PD0166285 (5 mg/kg) and AZD1775 (20 mg/kg) were administered *i.v.* in DMSO. Blood was collected by cardiac puncture 1 h after injection under isoflurane anesthesia, followed by brain tissue collection. Plasma was obtained by centrifugation (5 min, 5000 rpm, 4 °C). Brains were weighed and homogenized using a FastPrep®-24 (MP-Biomedicals, Santa Ana, CA) in 1% (*w*/*v*) bovine serum albumin in water. All samples were stored at −20 °C until analysis. AZD1775 and PD0166285 were extracted using diethyl ether and AZD8055 was used as internal standard. Organic phases were separated and dried by vacuum. Samples were reconstituted in methanol:water (20:80 *v*/v) and measured in an LC-MS/MS setup consisting of an Ultimate 3000 LC System (Dionex) and an API 4000 mass spectrometer (AB Sciex, Framingham, MA). Separation was performed on a ZORBAX Extend-C18 column (Agilent Technologies, Santa Clara, CA). Mobile phase A (0.1% formic acid in water) and B (methanol) was used in a 5 min gradient from 30 to 95%B maintained for 3 min followed by re-equilibration at 30%B. Multiple reaction monitoring (MRM) ion traces were 501.5 / 442.4 (AZD1775) and 512.2 / 438.9 (PD0166285) and 466.2 / 450.1 (AZD8055). Data were acquired and analyzed using Analyst® 1.6.2 software (AB Sciex).

Statistical analysis

CETA results were analyzed as described previously [18]. In short, the data was grouped by defining four sampling time points (30 min, 1 h, 2 h and 4 h) as a four-level within-subjects factor. Then, the general linear model repeated measures procedure of SPSS (v20; SPSS Inc., Chicago, IL) was used to determine whether the apical-basal differences were significantly increased by the factor of time. For *in vivo* pharmacokinetic experiments, one-way analysis of variance and post hoc Bonferroni was performed. In all experiments, differences were considered statistically significant when $p < 0.05$.

Results

P-gp and BCRP transport AZD1775 *in vitro*

Transport affinity of AZD1775 for P-gp and BCRP was first investigated *in vitro* using concentration equilibrium transport assays (CETAs). AZD1775 is clearly transported by both P-gp and BCRP (Fig. 2). First, an increasing difference in AZD1775 concentration over time between the apical and basolateral compartment was observed in both ABCG2 and Abcg2-expressing cell lines. Notably, this translocation also occurred in the MDCK parental cell line. Inhibition of translocation in all BCRP-expressing cell lines by elacridar confirmed that BCRP was responsible for this translocation, although full inhibition of transport was not achieved in the Abcg2-expressing cell line. This observation could be indicative of highly efficient AZD1775 transport by Abcg2, since full inhibition could be achieved in the endogenous canine BCRP expressing parental cell line and in the human BCRP-expressing cell line.

AZD1775 transport assays with cell lines expressing P-gp yielded similar results as those using BCRP-expressing lines. AZD1775 translocation was found in all cell lines expressing P-gp, including the parental cell line that expresses endogenous porcine P-gp. Intriguingly, transport could be inhibited by the P-gp inhibitor zosuquidar in the Abcb1a and ABCB1-expressing cell line, but not in the parental line, possibly indicating the presence of an additional unknown apically oriented transporter with substrate affinity for AZD1775 in this cell line.

PD0166285 is transported by P-gp, but not BCRP, *in vitro*

CETAs investigating translocation of PD0166285 revealed transport activity of P-gp, but not BCRP, *in vitro*. In none of the BCRP-expressing cell lines, translocation was observed (Fig. 3). In contrast to BCRP, both Abcb1a and ABCB1-expressing cell lines were found to transport PD0166285 while the parental porcine cell line was not. Again, loss of translocation in presence of the P-gp inhibitor

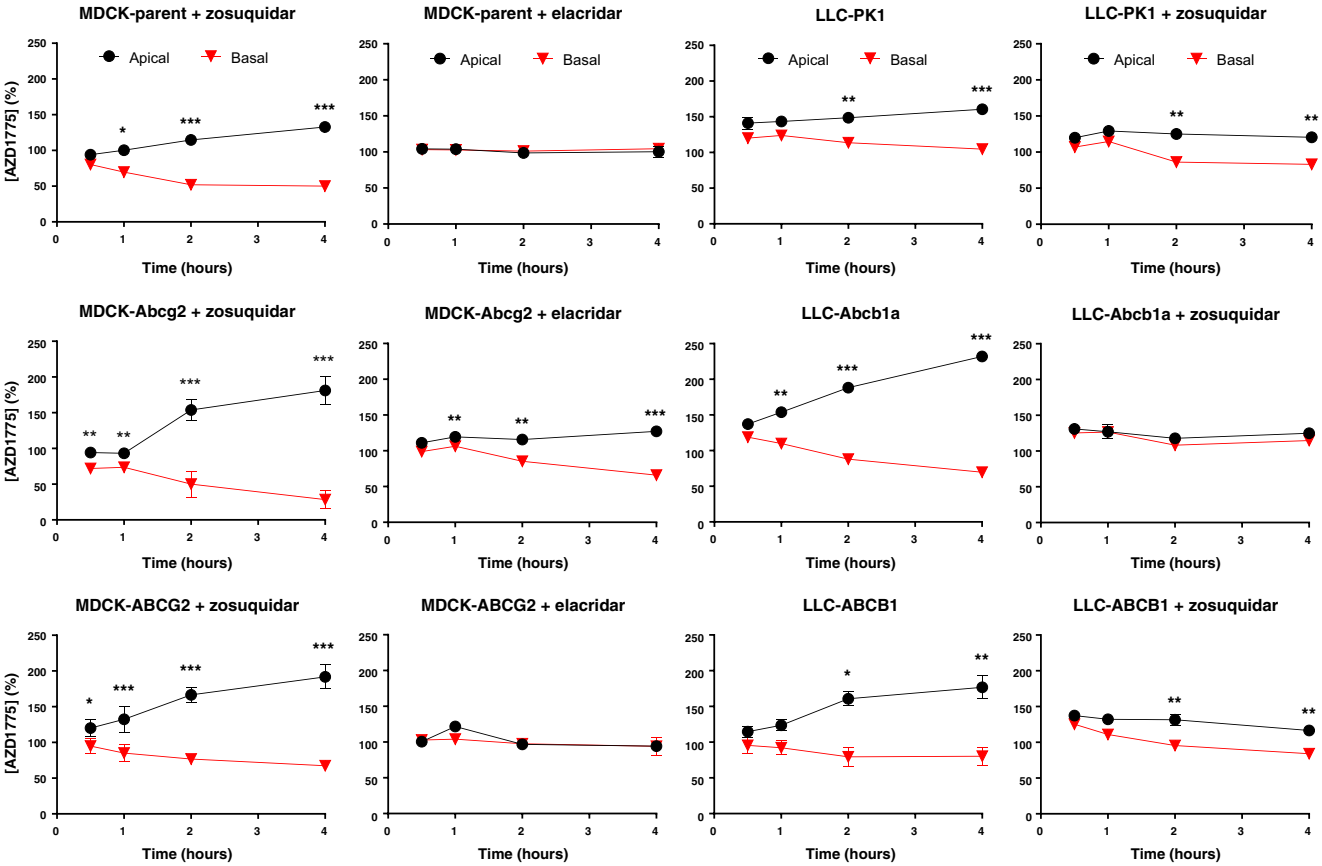

Fig. 2 In vitro transport of AZD1775 by P-gp and BCRP. Concentration equilibrium transport assays (CETAs) with various cell lines expressing murine or human P-gp or BCRP. AZD1775 showed profound basal to apical (B-to-A) translocation by Abcg2 and ABCG2. Interestingly, transport also occurred in the parental canine cell line. Note that any activity of endogenous canine P-gp in the MDCK lines was inhibited by adding 5 μM of zosuquidar. In all cell lines, BCRP transport was confirmed by inhibition with 5 μM of the BCRP/P-gp inhibitor elacridar. AZD1775 was transported by Abcb1a and ABCB1, which could be inhibited by 5 μM of the P-gp inhibitor zosuquidar. Some residual B-to-A translocation of AZD1775 was observed in the LLC-PK1 and LLC-ABCB1 that was not fully inhibited by 5 μM zosuquidar. Data are represented as mean ± SD ($n \geq 3$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

zosuquidar is a further confirmation that P-gp was responsible for the observed PD0166285 transport.

P-gp and BCRP work in concert to limit the brain penetration of AZD1775 *in vivo*

The impact of the ABC transporters P-gp and BCRP on the brain penetration of AZD1775 was tested in a pharmacokinetic experiment using mouse strains that were genetically engineered to lack one or multiple transporters. Intravenous administration of 20 mg/kg AZD1775 resulted in major differences in brain levels between ABC-transporter knockout mice 1 h after injection, while the plasma levels were similar among all strains (Fig. 4a). Compared to wild type control mice the AZD1775 brain concentration was elevated in Abcb1a/b$^{-/-}$ but not in Abcg2$^{-/-}$mice, suggesting that P-gp is the most dominant transporter limiting AZD1775 brain penetration. However, a further 6-fold increase in AZD1775 brain-plasma ratio that was observed in Abcb1a/b;Abcg2$^{-/-}$ compared to Abcb1a/b$^{-/-}$ mice demonstrates an important role for BCRP in AZD1775 efflux transport in the BBB *in vivo*. The presence of either one of these transporters is sufficient to cause a major reduction in the brain penetration of AZD1775.

P-gp, but not BCRP, limits the brain penetration of PD0166285 *in vivo*

A similar pharmacokinetic experiment as described above for AZD1775 was conducted investigating the brain penetration of PD0166285. In this experiment, approximately 5-fold increased brain levels were observed in both Abcb1a/b$^{-/-}$ and Abcb1a/b;Abcg2$^{-/-}$ mice compared to wild type mice (Fig. 4b). Thus, this effect appeared to be solely caused by P-gp, since further elevated PD0166285 brain levels were not observed when Abcg2$^{-/-}$ was also absent. These differences in brain levels were also reflected in the brain-plasma ratios since plasma levels were similar in all genetic backgrounds. In summary, these results indicate that the brain penetration of PD0166285 *in vivo* is limited by P-gp, but not BCRP.

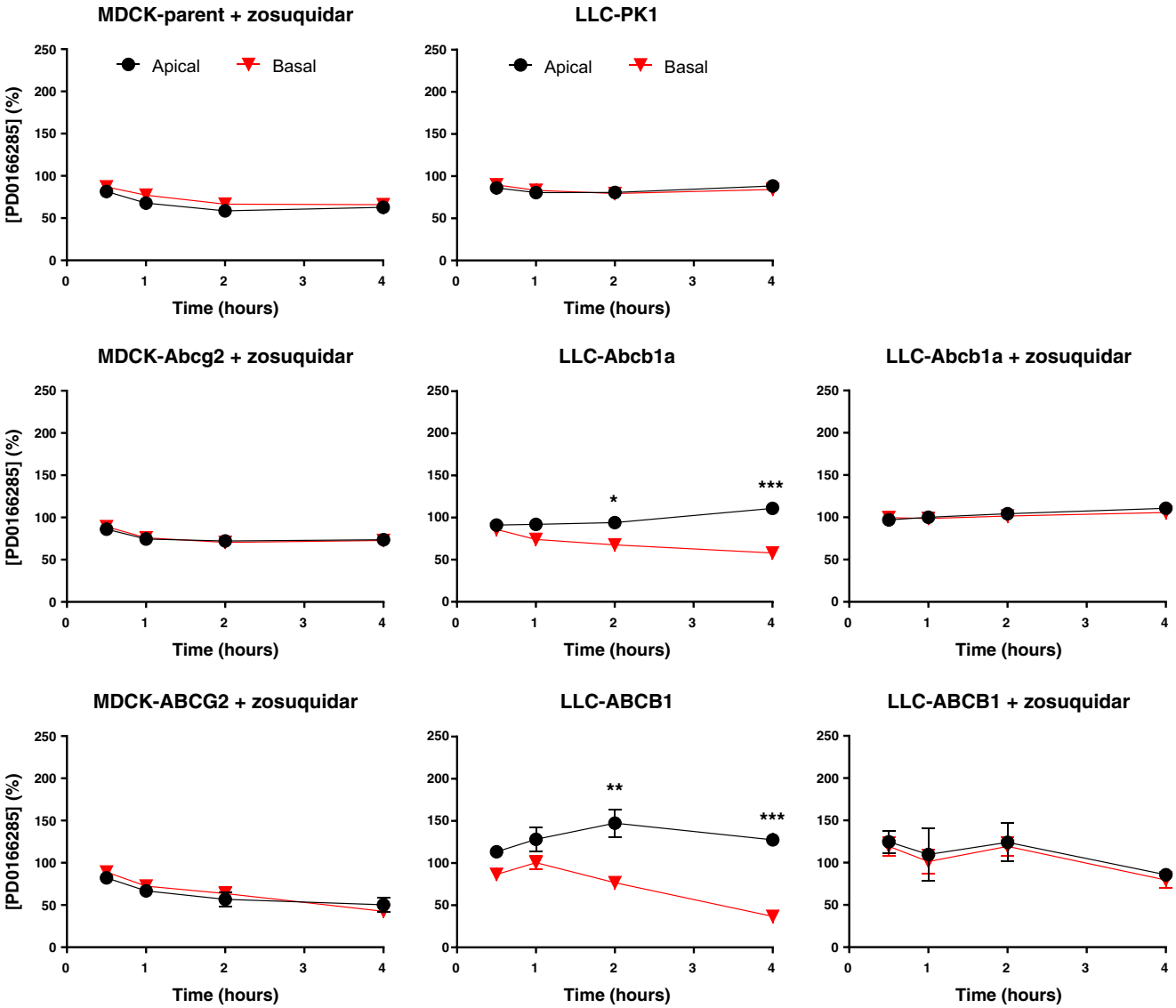

Fig. 3 In vitro transport of PD0166285 by P-glycoprotein. Concentration equilibrium transport assays (CETAs) with various cell lines expressing murine or human P-gp or BCRP. PD0166285 was transported by both Abcb1a and ABCB1, which was inhibited by 5 μM of the P-gp inhibitor zosuquidar. PD0166285 was not transported by Abcg2 or ABCG2. No transport was observed in the parental porcine cell line. Data are represented as mean ± SD (n ≥ 3); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Discussion

This set of *in vitro* and *in vivo* studies together demonstrate that the brain penetration of the Wee1 inhibitors AZD1775 and PD0166285 is strongly restricted by ABC transporters. First, *in vitro* transports assays demonstrate that AZD1775 is a substrate for both P-gp and BCRP, and that PD0166285 is a substrate for P-gp (Figs. 2 and 3). Next, pharmacokinetic experiments using wildtype and ABC transport knockout mice sampled for brain and plasma at 1 h after drug administration clearly show that these same transporters are responsible for the very low brain penetration of the Wee1 inhibitors *in vivo* (Fig. 4). Notably, in the absence of these transporters the brain-plasma ratio of both agents was remarkably high (approximately 25 for AZD1775 and 6 for PD0166285), whereas in wild type mice AZD1775 and PD0166285 could only achieve a brain-plasma ratio of 1.0 and 1.2, respectively.

The finding of this study that AZD1775 is efficiently transported by P-gp and BCRP in the blood-brain barrier could explain the previous report by Pokorny et al. demonstrating that AZD1775 exhibits heterogeneous penetration in an preclinical orthotopic brain tumor model [27]. Importantly, these authors have demonstrated that this heterogeneous brain tumor distribution limits antitumor efficacy in orthotopic glioma models, since efficacy could be observed against ectopic tumors. PD0166285 has not yet been investigated in a similar fashion, but the finding that it is an efficient P-gp substrate

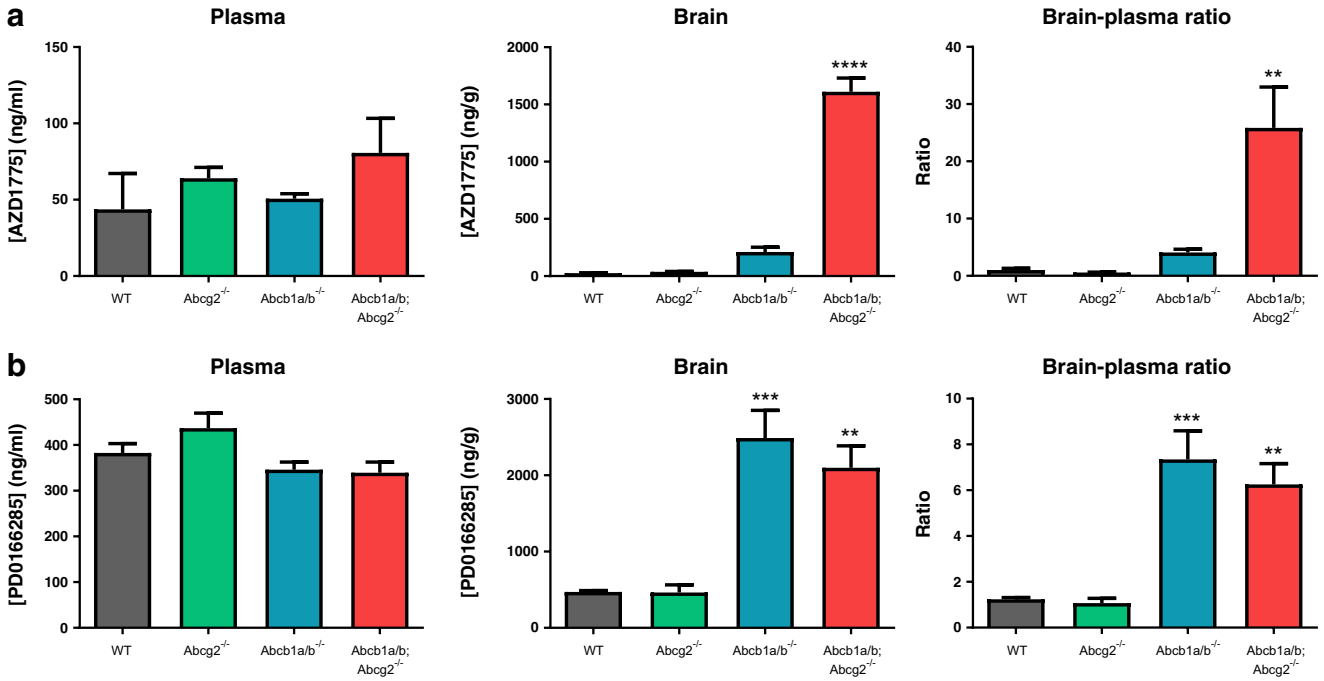

Fig. 4 The impact of P-gp and BCRP on the brain penetration of Wee1 inhibitors. **a** WT FVB, Abcg2$^{-/-}$, Abcb1a/b$^{-/-}$ and Abcb1a/b;Abcg2$^{-/-}$ mice were intravenously injected with 20 mg/kg AZD1775. After one hour, brain and plasma levels were determined and brain-plasma ratios were calculated. In contrast to Abcg2$^{-/-}$ mice, brain levels in Abcb1a/b$^{-/-}$ mice were elevated compared to WT mice while plasma levels were similar in all strains. Compared to Abcb1a/b$^{-/-}$ mice, brain levels of AZD1775 were even further increased in Abcb1a/b;Abcg2$^{-/-}$ mice, indicating that P-gp and BCRP work in concert to limit brain penetration of AZD1775. **b** Plasma concentrations, brain concentrations and brain-plasma ratios were determined in various ABC transporter knockout mice 1 h after i.v. administration of 5 mg/kg PD0166285. In contrast to Abcg2$^{-/-}$ mice, brain levels in Abcb1a/b$^{-/-}$ mice were elevated compared to WT mice at both time points while plasma levels were similar in all backgrounds. Compared to Abcb1a/b$^{-/-}$ mice, brain levels of PD0166285 were not further increased in Abcb1a/b;Abcg2$^{-/-}$ mice, indicating that P-gp, but not BCRP, limits the brain penetration of PD0166285. All data are represented as mean ± SD ($n \geq 4$); * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$

seems to predict that heterogeneous brain tumor distribution may likewise be found for PD0166285.

In contrast, a recent study by Wu et al. determined the brain-plasma ratio of AZD1775 in GBM patients by LC-MS/MS and found a brain-plasma ratio of approximately 7.5 [28]. This ratio is about 7-fold higher that the ratio reported in the present study and also seems to contradict the study by Pokorny et al. However, in the study by Wu et al. the brain-plasma ratio was measured in biopsies from the main mass of the brain tumor that is often leaky and thus might overestimate the general brain penetration [16]. Interestingly, the AZD1775 brain-plasma ratio in clinical specimens of the main tumor mass was similar to the brain-plasma ratio in Abcb1a/b;Abcg2$^{-/-}$ mice reported here, suggesting that the BBB was severely compromised in the regions of the tumor from which samples were collected by Wu et al.

Even though the core of a GBM tumor is leaky, the BBB still remains an important hurdle to successful GBM treatment. Glioma is a disease that is systemic to the brain as these tumor cells invade into normal surrounding brain structures where they find shelter behind an intact BBB [16]. Since the leaky core of the tumor is often removed by surgery, adjuvant therapies should especially target those migrated cells. There is a strong biological rationale for a role of Wee1 inhibitors in such a systemic therapy, but these inhibitors thus need to overcome the BBB and its ABC transporters. In this light, PD0166285 might be a better candidate than AZD1775. Even though both compounds are substrates of P-gp, PD0166285 is not transported by BCRP. This difference might be more important in patients than in mouse models, since BCRP is more abundant in human brain endothelial cells than in murine brain [29]. Moreover, the therapy-resistant glioma stem-cell like cell (GSC) compartment is also protected by expression of BCRP [30].

A disadvantage of PD0166285 compared to AZD1775 may be its lower selectivity. PD0166285 has originally been described to not only inhibit Wee1, but also MYT1 and CHK1 at approximately equimolar potency [5]. AZD1775 on the other hand was developed as a more specific Wee1 inhibitor, although recent reports suggest that AZD1775 also targets PLK1 [31, 32]. However, since all these kinases fulfill overlapping and complimentary roles in the G2 checkpoint [3], more detailed mechanistic studies should shed light on the true disadvantage of this relative aspecificity.

Obviously, the ideal Wee1 inhibitor for treatment of intracranial neoplasms combines high target specificity and no affinity for P-gp and BCRP. However, until such a candidate is developed, alternative approaches should be investigated in an effort to make Wee1 inhibitors available for treatment of brain tumors. One such strategy involves combined administration with a P-gp/BCRP inhibitor such as elacridar [33]. Unfortunately however, AZD1775 doesn't appear to be the most obvious candidate to investigate this approach in a clinical setting, because of its strong affinity for BCRP and the relative abundance of BCRP in the human BBB [29]. PD0166285 might be more promising for this treatment strategy, and our data suggest that this Wee1 inhibitor would potentially not need to be combined with a dual P-gp/BCRP inhibitor but could be investigated in combination with a potent P-gp inhibitor such as zosuquidar or tariquidar [34, 35].

In summary, targeting Wee1 to treat intracranial neoplasms holds promise since Wee1 is overexpressed in various glioma types and several clinical trials have been started. However, since gliomas are highly invasive and thus to a considerable extent protected by the BBB, using a Wee1 inhibitor with sufficient brain penetration capacity is pivotal to the success of this treatment strategy. We demonstrate that both available Wee1 inhibitors, AZD1775 and PD0166285, are efficient substrates of ABC transporters in the BBB and it is therefore not very likely that they will be able to exhibit efficacy in patients.

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Compliance with ethical standards

Conflict of interest Author Mark de Gooijer declares he has no conflicts of interest. Author Levi Buil declares he has no conflicts of interest. Author Jos H. Beijnen declares he has no conflicts of interest. Author Olaf van Tellingen declares he has no conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

References

1. Malumbres M, Barbacid M (2009) Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer 9(3):153–166. https://doi.org/10.1038/nrc2602
2. Medema RH, Macurek L (2012) Checkpoint control and cancer. Oncogene 31(21):2601–2613. https://doi.org/10.1038/onc.2011.451
3. de Gooijer MC, van den Top A, Bockaj I, Beijnen JH, Würdinger T, van Tellingen O (2017) The G2 checkpoint—a node-based molecular switch. FEBS Open Bio 7(4):439–455. https://doi.org/10.1002/2211-5463.12206
4. Dixon H, Norbury CJ (2002) Therapeutic exploitation of checkpoint defects in cancer cells lacking p53 function. Cell Cycle 1(6):362–368. https://doi.org/10.4161/cc.1.6.257
5. Wang Y, Li J, Booher RN, Kraker A, Lawrence T, Leopold WR, Sun Y (2001) Radiosensitization of p53 mutant cells by PD0166285, a novel G2 checkpoint abrogator. Cancer Res 61(22):8211–8217
6. Hirai H, Iwasawa Y, Okada M, Arai T, Nishibata T, Kobayashi M, Kimura T, Kaneko N, Ohtani J, Yamanaka K, Itadani H, Takahashi-Suzuki I, Fukasawa K, Oki H, Nambu T, Jiang J, Sakai T, Arakawa H, Sakamoto T, Sagara T, Yoshizumi T, Mizuarai S, Kotani H (2009) Small-molecule inhibition of Wee1 kinase by MK-1775 selectively sensitizes p53-deficient tumor cells to DNA-damaging agents. Mol Cancer Ther 8(11):2992–3000. https://doi.org/10.1158/1535-7163.mct-09-0463
7. Rajeshkumar NV, De Oliveira E, Ottenhof N, Watters J, Brooks D, Demuth T, Shumway SD, Mizuarai S, Hirai H, Maitra A, Hidalgo M (2011) MK-1775, a potent Wee1 inhibitor, synergizes with gemcitabine to achieve tumor regressions, selectively in p53-deficient pancreatic cancer xenografts. Clin Cancer Res 17(9):2799–2806. https://doi.org/10.1158/1078-0432.ccr-10-2580
8. Bridges KA, Hirai H, Buser CA, Brooks C, Liu H, Buchholz TA, Molkentine JM, Mason KA, Meyn RE (2011) MK-1775, a novel Wee1 kinase inhibitor, radiosensitizes p53-defective human tumor cells. Clin Cancer Res 17(17):5638–5648. https://doi.org/10.1158/1078-0432.ccr-11-0650
9. Vriend LE, De Witt Hamer PC, Van Noorden CJ, Wurdinger T (2013) WEE1 inhibition and genomic instability in cancer. Biochim Biophys Acta 1836(2):227–235. https://doi.org/10.1016/j.bbcan.2013.05.002
10. Cuneo KC, Morgan MA, Davis MA, Parcels LA, Parcels J, Karnak D, Ryan C, Liu N, Maybaum J, Lawrence TS (2016) Wee1 kinase inhibitor AZD1775 radiosensitizes hepatocellular carcinoma regardless of TP53 mutational status through induction of replication stress. Int J Radiat Oncol Biol Phys 95(2):782–790. https://doi.org/10.1016/j.ijrobp.2016.01.028
11. Puigvert JC, Sanjiv K, Helleday T (2016) Targeting DNA repair, DNA metabolism and replication stress as anti-cancer strategies. FEBS J 283(2):232–245. https://doi.org/10.1111/febs.13574
12. Leijen S, van Geel RMJM, Sonke GS, de Jong D, Rosenberg EH, Marchetti S, Pluim D, van Werkhoven E, Rose S, Lee MA, Freshwater T, Beijnen JH, Schellens JHM (2016) Phase II study of WEE1 inhibitor AZD1775 plus carboplatin in patients with TP53-mutated ovarian cancer refractory or resistant to first-line therapy within 3 months. J Clin Oncol 34(36):4354–4361. https://doi.org/10.1200/jco.2016.67.5942
13. Mir SE, De Witt Hamer PC, Krawczyk PM, Balaj L, Claes A, Niers JM, Van Tilborg AA, Zwinderman AH, Geerts D, Kaspers GJ, Peter Vandertop W, Cloos J, Tannous BA, Wesseling P, Aten JA, Noske DP, Van Noorden CJ, Wurdinger T (2010) In silico analysis of kinase expression identifies WEE1 as a gatekeeper against mitotic catastrophe in glioblastoma. Cancer Cell 18(3):244–257. https://doi.org/10.1016/j.ccr.2010.08.011
14. De Witt Hamer PC, Mir SE, Noske D, Van Noorden CJ, Wurdinger T (2011) WEE1 kinase targeting combined with DNA-damaging cancer therapy catalyzes mitotic catastrophe. Clin Cancer Res 17(13):4200–4207. https://doi.org/10.1158/1078-0432.CCR-10-2537
15. Caretti V, Hiddingh L, Lagerweij T, Schellen P, Koken PW, Hulleman E, van Vuurden DG, Vandertop WP, Kaspers GJ, Noske DP, Wurdinger T (2013) WEE1 kinase inhibition enhances the radiation response of diffuse intrinsic pontine gliomas. Mol Cancer Ther 12(2):141–150. https://doi.org/10.1158/1535-7163.MCT-12-0735

16. van Tellingen O, Yetkin-Arik B, de Gooijer MC, Wesseling P, Wurdinger T, de Vries HE (2015) Overcoming the blood-brain tumor barrier for effective glioblastoma treatment. Drug Resist Updat 19:1–12. https://doi.org/10.1016/j.drup.2015.02.002
17. Durmus S, Hendrikx JJMA, Schinkel AH (2015) Apical ABC transporters and cancer chemotherapeutic drug disposition. Adv Cancer Res 125:1–41. https://doi.org/10.1016/bs.acr.2014.10.001
18. de Gooijer MC, Zhang P, Thota N, Mayayo-Peralta I, Buil LC, Beijnen JH, van Tellingen O (2015) P-glycoprotein and breast cancer resistance protein restrict the brain penetration of the CDK4/6 inhibitor palbociclib. Investig New Drugs 33(5):1012–1019. https://doi.org/10.1007/s10637-015-0266-y
19. Oberoi RK, Mittapalli RK, Elmquist WF (2013) Pharmacokinetic assessment of efflux transport in sunitinib distribution to the brain. J Pharmacol Exp Ther 347(3):755–764. https://doi.org/10.1124/jpet.113.208959
20. Lin F, de Gooijer MC, Roig EM, Buil LC, Christner SM, Beumer JH, Wurdinger T, Beijnen JH, van Tellingen O (2014) ABCB1, ABCG2, and PTEN determine the response of glioblastoma to temozolomide and ABT-888 therapy. Clin Cancer Res 20(10):2703–2713. https://doi.org/10.1158/1078-0432.CCR-14-0084
21. Parrish KE, Pokorny J, Mittapalli RK, Bakken K, Sarkaria JN, Elmquist WF (2015) Efflux transporters at the blood-brain barrier limit delivery and efficacy of cyclin-dependent kinase 4/6 inhibitor palbociclib (PD-0332991) in an orthotopic brain tumor model. J Pharmacol Exp Ther 355(2):264–271. https://doi.org/10.1124/jpet.115.228213
22. Lin F, de Gooijer MC, Hanekamp D, Chandrasekaran G, Buil LCM, Thota N, Sparidans RW, Beijnen JH, Würdinger T, van Tellingen O (2017) PI3K–mTOR pathway inhibition exhibits efficacy against high-grade glioma in clinically relevant mouse models. Clin Cancer Res 23(5):1286–1298. https://doi.org/10.1158/1078-0432.ccr-16-1276
23. Becker CM, Oberoi RK, McFarren SJ, Muldoon DM, Pafundi DH, Pokorny JL, Brinkmann DH, Ohlfest JR, Sarkaria JN, Largaespada DA, Elmquist WF (2015) Decreased affinity for efflux transporters increases brain penetrance and molecular targeting of a PI3K/mTOR inhibitor in a mouse model of glioblastoma. Neuro-Oncology 17(9):1210–1219. https://doi.org/10.1093/neuonc/nov081
24. Schinkel AH, Wagenaar E, van Deemter L, Mol CA, Borst P (1995) Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin. A J Clin Invest 96(4):1698–1705. https://doi.org/10.1172/JCI118214
25. Jonker JW, Smit JW, Brinkhuis RF, Maliepaard M, Beijnen JH, Schellens JHM, Schinkel AH (2000) Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. J Natl Cancer Inst 92(20):1651–1656. https://doi.org/10.1093/jnci/92.20.1651
26. Pavek P, Merino G, Wagenaar E, Bolscher E, Novotna M, Jonker JW, Schinkel AH (2005) Human breast cancer resistance protein: interactions with steroid drugs, hormones, the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine, and transport of cimetidine. J Pharmacol Exp Ther 312(1):144–152. https://doi.org/10.1124/jpet.104.073916
27. Pokorny JL, Calligaris D, Gupta SK, Iyekegbe DO, Mueller D, Bakken KK, Carlson BL, Schroeder MA, Evans DL, Lou Z, Decker PA, Eckel-Passow JE, Pucci V, Ma B, Shumway SD, Elmquist WF, Agar NYR, Sarkaria JN (2015) The efficacy of the Wee1 inhibitor MK-1775 combined with temozolomide is limited by heterogeneous distribution across the blood–brain barrier in glioblastoma. Clin Cancer Res 21(8):1916–1924. https://doi.org/10.1158/1078-0432.ccr-14-2588
28. Wu J, Sanai N, Bao X, LoRusso P, Li J (2016) An aqueous normal-phase chromatography coupled with tandem mass spectrometry method for determining unbound brain-to-plasma concentration ratio of AZD1775, a Wee1 kinase inhibitor, in patients with glioblastoma. J Chromatogr B Anal Technol Biomed Life Sci 1028:25–32. https://doi.org/10.1016/j.jchromb.2016.05.050
29. Uchida Y, Ohtsuki S, Katsukura Y, Ikeda C, Suzuki T, Kamiie J, Terasaki T (2011) Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors. J Neurochem 117(2):333–345. https://doi.org/10.1111/j.1471-4159.2011.07208.x
30. Bleau AM, Hambardzumyan D, Ozawa T, Fomchenko EI, Huse JT, Brennan CW, Holland EC (2009) PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. Cell Stem Cell 4(3):226–235. https://doi.org/10.1016/j.stem.2009.01.007
31. Wright G, Golubeva V, Remsing Rix LL, Berndt N, Luo Y, Ward GA, Gray JE, Schonbrunn E, Lawrence HR, Monteiro ANA, Rix U (2017) Dual targeting of WEE1 and PLK1 by AZD1775 elicits single agent cellular anticancer activity. ACS Chem Biol 12(7):1883–1892. https://doi.org/10.1021/acschembio.7b00147
32. Zhu J-Y, Cuellar RA, Berndt N, Lee HE, Olesen SH, Martin MP, Jensen JT, Georg GI, Schönbrunn E (2017) Structural basis of wee kinases functionality and inactivation by diverse small molecule inhibitors. J Med Chem 60(18):7863–7875. https://doi.org/10.1021/acs.jmedchem.7b00996
33. Hyafil F, Vergely C, Du Vignaud P, Grand-Perret T (1993) *In vitro* and *in vivo* reversal of multidrug resistance by GF120918, an acridonecarboxamide derivative. Cancer Res 53(19):4595–4602
34. Dantzig AH, Shepard RL, Cao J, Law KL, Ehlhardt WJ, Baughman TM, Bumol TF, Starling JJ (1996) Reversal of P-glycoprotein-mediated multidrug resistance by a potent cyclopropyldibenzosuberane modulator, LY335979. Cancer Res 56(18):4171–4179
35. Martin C, Berridge G, Mistry P, Higgins C, Charlton P, Callaghan R (1999) The molecular interaction of the high affinity reversal agent XR9576 with P-glycoprotein. Br J Pharmacol 128(2):403–411. https://doi.org/10.1038/sj.bjp.0702807