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Pharmacokinetic Properties of Anticancer Agents for the Treatment of CNS Tumors: Update of the Literature

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Abstract

Despite significant improvement in outcomes for patients with hematological malignancies and solid tumors over the past 10 years, patients with primary or metastatic brain tumors continue to have a poor prognosis. A primary reason for this is the inability of many chemotherapeutic drugs to penetrate into the brain and brain tumors at concentrations high enough to exert an antitumor effect due to unique barriers and efflux transporters. Several studies have been published recently examining the CNS pharmacokinetics of various anticancer drugs in patients with primary and metastatic brain tumors. To summarize recent advances in the field, this review will critically present studies published within the last 9 years examining brain and cerebrospinal fluid penetration of clinically available anticancer agents for patients with CNS tumors.

1. Introduction

Despite advances in the therapy of many cancers, central nervous system (CNS) tumors are one of the most challenging malignancies to treat, accounting for a large proportion of cancer-related deaths in adults and children. From 1995–2011, the 5-year survival rates ranged from ~5% for patients with glioblastoma to ~83% for patients with ependymal tumors, with little change in survival during this time period [1]. In children less than 19 years old, malignant brain tumors are the leading cause of cancer death [1]. The 5-year survival rate for pediatric CNS tumors varies widely dependent upon tumor type with patients with glioblastoma tumors having an 18% survival rate compared with patients with pilocytic astrocytoma who have a 97% survival rate [1]. In addition to primary CNS tumors, the frequency of CNS metastases in patients with hematological malignancies and solid tumors is increasing and treatment options for these patients remain limited [2]. The incidence and unfavorable prognosis of patients with primary CNS tumor and CNS metastases highlight the need for development of new and more successful therapies. It is imperative that researchers in the field of drug development for these indications incorporate knowledge on the unique pharmacokinetic (PK) properties required for CNS drug penetration along with biological properties of the disease. Although many studies have

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been published describing the CNS pharmacokinetics of drugs used to treat brain tumors, interpretation of the results can be ambiguous due to varying study designs and methodologies. Thus, the objective of this review is to provide a comprehensive and critical assessment of PK studies from 2006-present that have examined the CNS distribution of drugs used for treatment of primary CNS tumors or CNS metastases (Table I).

2. Barriers to anticancer agent penetration into the brain

2.1 Physical barriers

2.1.1 Blood brain barrier—The blood brain barrier (BBB) is a unique anatomical structure that effectively regulates passage of molecules into the CNS due to the complex interactions of endothelial cells, astrocytes, pericytes, transporters, and the extracellular matrix [3]. Although a diagram of the BBB is not provided in this manuscript, many reviews of the BBB include excellent figures of the brain/spinal physiological spaces and we point the reader to these references [3–7]. Both physiochemical and biological processes control the transfer of molecules across this barrier. Most lipophilic and certain hydrophilic compounds (depending on physiochemical properties) undergo transmembrane diffusion while the majority of hydrophilic compounds are transported by carrier mediated transport processes [8]. Large molecules such as insulin and transferrin undergo transport by receptor mediated endocytosis and other large molecules such as albumin or other plasma proteins undergo adsorptive endocytosis [8].

Small molecules are often believed to penetrate the BBB and a limited number can cross the barrier by transcellular or paracellular transport. However, several factors prevent greater penetration including BBB anatomy, efflux transporters present at the BBB, and physiochemical properties of the molecule [9]. The ideal CNS therapeutic agent is generally < 400 g/mol, has a high lipophilicity ($\log P \sim 2.5$), is nonpolar, not ionized, has low hydrogen bond capacity, is not a substrate for efflux transporters, and has a low level of protein binding [10].

2.1.2 Blood-cerebrospinal fluid barrier—The blood-cerebrospinal fluid (CSF) barrier (BCSFB) separates the blood from the CSF at the choroid plexus and the outer arachnoid membrane [8]. The choroid plexus, responsible for the majority of CSF production, contains fenestrated, highly permeable capillaries at the blood facing side surrounded by a monolayer of tight junction forming epithelial (ependymal) cells facing the CSF side. Drug molecules in the systemic circulation arriving at the BCSFB may pass through capillary fenestrations, but penetration of the additional ependymal layer must be accomplished before the drug can reach the CSF [8].

Unbound drug concentrations at the site of action are assumed to be the best predictor for pharmacodynamic effect and the CSF has been suggested as a surrogate for targets close to the ventricles. However, the common assumption that transport across the BCSFB is indicative of uptake into the brain parenchyma is misguided for several reasons. Drug concentrations across the brain can vary by orders of magnitude depending on the location of drug entry into the CSF. This is best illustrated by higher drug concentrations in the lumbar CSF compared to ventricular CSF concentrations after intravenous or intrathecal

drug administration [11, 12]. Additionally, the presence of efflux transporters and tight junctions differentiate the BBB and BCSFB from other physiological membranes, which can result in unbound drug concentrations being different in the CSF than in the brain or tumor ECF [13]. Indeed, preclinical studies have shown unbound drug in the CSF to be higher than brain ECF concentrations under presumed steady-state conditions and a clinical study showed similar results [14, 15]. Another consideration is CSF turnover, which occurs four to five times each day, resulting in a bulk flow of CSF from the CNS to systemic circulation yielding lower CSF drug concentrations [16]. Lastly, drugs entering the CSF via transport across the BCSFB are often rapidly exported back to systemic circulation due to absorption by arachnoid villi or by bulk flow rates of CSF back into systemic circulation [17]. However, direct measurement of brain tissue concentrations clinically is uncommon and despite the discrepancies, CSF sampling is a more feasible strategy for investigating CNS drug concentrations.

2.1.3 Blood-brain tumor barrier—The blood-brain tumor barrier (BBTB) is formed by a heterogeneous group of native brain and foreign tumor capillaries and is suggested to be leakier than the BBB based on contrast-enhanced magnetic resonance imaging (MRI) [18–20]. Groothuis et al., described distinct microvessel populations that vary in permeability from continuous, nonfenestrated capillaries similar to normal brain tissue to the presence of wide inter-endothelial gaps resulting in permeability to large molecules [21]. The wide gaps between endothelial cells would suggest an impaired BBB that could increase tumor exposure to anticancer agents. However, this leakiness may be a local feature while the majority of the BBTB permeability parallels the BBB, preventing sufficient penetration of anticancer agents and pharmacodynamic effect (e.g. tumor inhibition) [22, 23]. This is supported by preclinical work in two models of breast cancer brain metastases, which showed that even when the BBTB is impaired, increasing permeability, the amount of drug reaching the tumor is still not sufficient to achieve a cytotoxic response. [24]. Thus, despite an impaired barrier in certain locations of the tumor, “leakiness” of the BBTB in general does not result in an increase in drug delivery sufficient for a pharmacodynamics response.

2.2 Efflux transporters

In addition to the physical barriers, active efflux transporters on the membranes of the BBB and BCSFB limit penetration of certain agents into the CNS. Several recent reviews have detailed the roles of transporters at the BBB and BCSFB [25, 26]. Briefly, P-glycoprotein (P-gp/ABCB1) is the most extensively studied ATP-binding cassette (ABC) transporter at both the BBB and BCSF barrier. P-gp is located on the luminal side of brain endothelial cells and thereby causes an efflux of anticancer agents that are substrates out of brain parenchyma, reducing the brain/plasma ratio of these agents and potentially leading to drug resistance [27]. P-gp expression on the apical side of choroid plexus ependymal cells causes an influx of substrates into the CSF, increasing the CSF/plasma ratios of these agents, highlighting the importance of knowing if drugs are substrates for P-gp when examining their CNS PK [28]. Breast-cancer related protein (BCRP/ABCG2) and multi-drug resistance proteins (MRPs) can alter the transport of anticancer agents in and out of brain tissue [29]. Expression of these transporters in microvessels within brain tumors such as ependymoma can limit the penetration of certain agents into tumors and have a dramatic effect on CNS

PK and pharmacodynamic (PD) properties [30]. Clinical trials using P-gp inhibitors to reduce drug resistance due to efflux and increase tumor penetration of anticancer agents have been unsuccessful largely due to poor compound potency (i.e., tolerable unbound systemic concentration much less than the K_i value), and increased adverse drug effects attributable to transporter inhibitor single agent treatment or the combination of the anticancer agent and the transporter inhibitor [31]. These results demonstrate the need for further development of more sophisticated transporter inhibitors to increase substrate penetration into the CNS.

3. CNS penetration of anticancer agents used to treat CNS tumors

3.1 Alkylating agents

Alkylating agents, widely used for the treatment of many neoplasms, exert their effect by substituting alkyl groups for hydrogen atoms on DNA leading to the formation of cross links [32]. This large group of compounds is commonly divided into six separate classes including mustards, nitrosoureas, tetrazines, aziridines, platinating agents, and non-classical alkylating agents. This review will highlight the two alkylating agents with recently published CNS PK: temozolomide (TMZ) and ifosfamide (IFO).

TMZ, an alkylating agent of the tetrazine class, is currently indicated for treatment of various brain tumors including astrocytoma and glioblastoma multiforme, and is in clinical trials for medulloblastoma and neuroblastoma [33, 34]. TMZ has a molecular weight of 194 g/mol, is 12–18% protein bound, and has been shown to penetrate the BBB [34, 35]. Although TMZ is not a P-gp substrate, results from a recent study show that TMZ can down-regulate P-gp expression in human BBB cells via disruption of Wnt3 signaling [36].

In a recent feasibility study, Portnow et al. used intracerebral microdialysis (ICMD) to evaluate the neuropharmacokinetics (nPK) of TMZ in seven patients with various types of CNS tumors [37]. TMZ was given as a single oral dose of 150 mg/mg² with plasma and dialysate samples from peritumoral brain interstitium (BI) collected at predetermined time points after dosing. The results showed a median ECF/plasma AUC ratio of 0.18 with unbound TMZ concentrations in the BI rising more gradually and staying elevated longer than total plasma TMZ concentrations. A preclinical PK model derived from TMZ brain concentrations obtained from non-tumor bearing rats predicted a human brain to plasma area under the curve (AUC) ratio of 0.2, which corresponds with the results from the human study [38]. The results are also similar to TMZ measured in CSF obtained via lumbar puncture (median CSF/plasma ratio 0.30; range, 0.02–0.65) [39]. While this study demonstrated that using ICMD to determine the nPK of TMZ is safe and feasible, certain limitations should be considered with regards to the measurement of TMZ concentrations. First, the ICMD probe was placed in peritumoral BI so the TMZ concentration measured may not be reflective of TMZ concentration in tumor tissue. Also, a common issue with some ICMD studies is the inability to perform *in vivo* recovery studies so the investigators use *in vitro* probe recovery to determine actual concentrations. However, *in vivo* recovery is typically lower than *in vitro* recovery values which may result in underestimation of actual concentrations in the BI.

In a separate study, positron emission tomography (PET) was used to examine the tissue distribution of TMZ in glioma patients [40]. The authors examined seven PET scans from recurrent glioma patients. Five patients received 75–200 mg/m²/d oral TMZ over 5 d every 28 d, and two patients received the same daily TMZ dosage for 6 to 7 weeks. PET scans were acquired over 90 min on the last day of the cycle approximately 6 h after the last TMZ dose and plasma samples were taken during the scans. PET imaging showed a heterogeneous uptake of [methyl-¹¹C] temozolomide into brain tumor tissue and suggested that the transport rate of TMZ into tumor tissue ($K_1=0.052$) was greater than into normal brain ($K_1=0.033$). Interestingly, while TMZ penetrated into the tumor tissue more rapidly and to a greater extent, it was also subsequently effluxed to the plasma more than in normal brain tissue, resulting in similar mean residence times (MRT) between the two tissues. The authors also used a convolution approach to predict the tissue distribution of non-radioactive TMZ under different clinical dosing schedules. Similar to radiolabeled TMZ, peak tumor concentrations were predicted to be higher than peak brain TMZ concentrations. Their model also predicted the ratios of tissue to plasma exposure were ~1.3 for glioma tumors and ~0.9 for normal brain. These ratios are significantly higher than those found using ICMD (0.18) and CSF (0.31) sampling. These differences may be due to: (1) the difference between total drug concentrations (from PET study) versus unbound concentrations (from ICMD study), and (2) the different methodologies used to collect samples and measure TMZ concentrations in brain or tumor tissue (PET vs. ICMD).

Ifosfamide (IFO), an alkylating agent of the mustard class, is used to treat a variety of malignancies including non-Hodgkin's lymphoma and primary CNS lymphoma in adults, and Ewing sarcoma in children [41–43]. IFO is a prodrug that undergoes hydroxylation by cytochrome P-450 isozymes to form the metabolite 4-hydroxy-ifosfamide (4-OH-IFO), which is unstable and spontaneously forms the highly active isophosphoramidate mustard and acrolein. IFO has a low molecular weight, demonstrates minimal protein binding, is not a substrate for efflux transporters and therefore it is thought to significantly penetrate the BBB [44].

Recently, Kiewe et al. examined the penetration of IFO and 4-OH-IFO into the CSF of patients with CNS malignancies [45]. Twelve adults were administered IFO 1,300–2,000 mg/m² over 3 h via IV infusion on days 3–5 in addition to high-dose methotrexate (4,000mg/m²) over 4 h on day 1. Plasma samples were collected before or immediately after IFO infusion and a single CSF sample via lumbar puncture was taken 10 min after the end of IFO infusion. For patients with measurable CSF concentrations, the median (range) IFO CSF/plasma and 4-OH-IFO CSF/plasma ratios were 0.38 (0.18–0.72) and 3.07 (0.62–29.12), respectively. Significant inter-patient variability was observed for both IFO and 4-OH-IFO, and no 4-OH-IFO was detected in 35% of the CSF samples collected. These results agree with previous studies reporting median CSF/plasma ratios for IFO metabolites that vary from 0 to 3.2, with high inter-patient variability that may explain differences in response [46–49]. In contrast to previous reports, however, no significant differences in IFO or 4-OH-IFO CSF penetration were observed in the presence of corticosteroids [48]. While the results from Kiewe et al. demonstrate that IFO and 4-OH-IFO penetrate into the CSF in a subset of patients, the large inter-patient variability, small sample size, and limited sampling strategy (non-serial sampling) used limit the applicability of these results to other clinical scenarios.

3.2 Nucleoside Analogues

Nucleoside analogs structurally resemble endogenous nucleosides and induce cytostatic or cytotoxic effects when incorporated into cellular DNA or RNA. Due to their relatively low molecular weight and structural identity with endogenous nucleosides, nucleoside analogs have a high probability for CNS penetration.

Gemcitabine (dFdC, 2', 2' - difluorodeoxycytidine) a pyrimidine nucleoside analog, is FDA-approved for treatment of various solid tumors such as ovarian, breast, non-small cell lung, and pancreatic cancers. Gemcitabine has been tested for treatment of various brain metastases in combination with other chemotherapy or targeted therapy [50–52], and is currently under investigation for treatment of pediatric medulloblastoma (NCT01878617). After intracellular transport, gemcitabine is enzymatically converted to the active metabolites gemcitabine di- or triphosphate by deoxycytidine kinase or the inactive metabolite difluorodeoxyuridine (dFdU) by cytidine deaminase. Gemcitabine pharmacology has been reviewed extensively in detail elsewhere [53]. Gemcitabine has a molecular weight of 263 g/mol, negligible protein binding, and is not a substrate for efflux transporters, making it an attractive candidate for treatment of CNS tumors.

In a phase I trial, Bernardi and colleagues examined gemcitabine CSF pharmacokinetics after intraventricular gemcitabine administration in eight pediatric and adult patients with neoplastic meningitis [54]. Gemcitabine was administered via lumbar puncture or Ommaya reservoir at a dose of 5 mg over 5 min once or twice per week, and plasma and CSF were sampled pre- and serially up to 24 h post-drug administration. Neither gemcitabine nor dFdU were detected in the plasma. The gemcitabine CSF half-life ($t_{1/2}$) was 61 ± 50 min and the maximal CSF concentration ($C_{\max}$) was 939 ± 384 μ M comparable to concentrations achieved in non-human primates (NHP) receiving 5 mg intraventricular gemcitabine [55]. However, due to 10-fold difference in CSF volume between two species (NHP < human), the 5 mg NHP dose is equivalent to 50 mg in human. This indicates that despite a comparable CSF $C_{\max}$, NHP exhibit a higher gemcitabine clearance compare to human. Consistent with lower gemcitabine clearance in humans, formation of the inactive metabolite, dFdU, was lower in human CSF (median gemcitabine/dFdU CSF AUC ratio ~ 0.011 , range 0.003 – 0.017) compared to NHP (median gemcitabine/dFdU CSF AUC ratio ~ 1.20) indicating a higher cytidine deaminase activity in NHP [55]. In a separate study in adult patients with recurrent glioblastoma multiforme, Sigmond et al measured gemcitabine and dFdU in plasma and tumor samples. Patients received 500 or 1000 mg/m² gemcitabine as 30 min infusion, and blood samples were collected before and 30 min after infusion, and at the time of tumor collection (1 to 4 h after gemcitabine infusion) [56]. Gemcitabine and dFdU concentrations in glioblastoma tumors were 0.06 to 3.58 nmol/g tissue and 29 to 72 nmol/g tissue, respectively. Gemcitabine and dFdU plasma concentrations at the time of tumor collection ranged from BQL (0.019 μ M) - 9.2 μ M and 24.8 – 42.7 μ M at the 500 mg/m² dosage and BQL - 3.4 μ M and 45.6 – 72.6 μ M at the 1000 mg/m² dosage, respectively. It is important to note that tumor samples were collected as biopsy specimens during surgery, and residual blood contamination could significantly contribute to tumor concentrations of gemcitabine and metabolites observed.

Capecitabine, a prodrug of the pyrimidine analog 5-fluorouracil (5-FU), is FDA approved for the treatment of colorectal and breast cancer and is currently in clinical trials with radiation therapy in pediatric glioma [57]. Capecitabine has a molecular weight of 359 g/mol, is < 60% protein bound, and based upon preclinical studies is poorly CNS penetrant [58].

Morikawa et al. studied the disposition of capecitabine and its metabolites in serum and breast cancer brain metastases from patients undergoing surgical resection who received a single oral dose of 1250 mg/m² capecitabine (n=8) given 2–3 h prior to surgery [59]. The median concentrations of capecitabine and 5-FU observed in tumors were 0.81, and 1.81 μM, respectively, whereas median tumor to serum concentration ratios at the time of tumor collection were 0.28 and 5.64, respectively. Large between and within patient variability was observed in the tumor concentrations of capecitabine and its metabolites.

3.3 Topotecan

Topotecan (TPT) is currently indicated for treatment of ovarian, cervical, and small cell lung cancer, and is in clinical trials for medulloblastoma and neuroblastoma [60]. TPT, a water-soluble camptothecin analog has an α-hydroxyl lactone system that at physiologic pH exists in equilibrium between the active lactone and the inactive hydroxyl acid form [61, 62]. TPT exerts its anticancer effect by interacting with the topoisomerase I-DNA complex and preventing religation of single stranded DNA breaks. Although TPT is a substrate for both P-gp and BCRP, it has been shown to penetrate the BBB and BCSFB [63, 64].

Morgan and colleagues measured plasma and CSF TPT concentrations in a phase I study of 15 patients receiving TPT as a 72-hour continuous infusion along with tamoxifen and carboplatin [65]. CSF samples were obtained from eligible patients via lumbar puncture (LP) immediately prior to the end of the TPT infusion during the first course of therapy. The mean peak total (i.e., lactone and carboxylate) TPT plasma concentration at the maximally tolerated dosage (0.75 mg/m²) was 5.98 nM, of which the TPT lactone was 1.18 nM. The total CSF TPT concentration observed at that dosage level was 1.66 nM, and the lactone CSF concentration was below the lower limit of quantitation (0.55 nM). The CSF lactone TPT was only measurable in one patient at the 1.0 mg/m² dosage level, and the value was 1.09 nM. The authors estimated the TPT CSF to plasma ratio for total TPT as 0.21, comparable to the values reported by Baker et al. in children with recurrent brain tumors receiving TPT as a 72-h continuous infusion [64].

Motivated by the need to identify agents that could be administered intrathecally to treat tumors found in the meninges, Blaney et al. performed a phase I study to determine the optimal dose of TPT when administered daily for five days [66]. Intraventricular concentrations were measured to determine whether the maximum tolerated dose of TPT was also the dose that maintained TPT lactone CSF concentrations > 2.18 nM for at least 8 h (the optimal dose). Patients received intraventricular TPT administered (0.1 or 0.2 mg/dose) during induction, consolidation, and then every 4 weeks during maintenance. Ventricular CSF PK studies were performed in 18 patients on day 1, week 1 of induction, and in six patients after a single intralumbar TPT dose on day 1, week 3 (0.1 or 0.2 mg/dose). After the day 1 dose of intraventricular TPT at 0.2 mg, the median (range) estimated time TPT CSF lactone concentrations were above 2.18 nM was 24 h (10.9 to >24 h). The TPT

concentration versus time data from the patients receiving intralumbar TPT was variable with median (range) time to maximum concentration and maximum concentration of 4.5 h (0.43 to 6.3 h) and 0.12 μ M (0.006 to 1.89 μ M), respectively. However, all patients had TPT lactone concentrations above 2.18 nM for >8 h after lumbar drug administration. Using Monte-Carlo simulations the authors predicted that at the maximum dose of 0.2 mg, all patients were predicted to have TPT lactone CSF concentrations > 2.18 nM for 8 h and 38% of patients were predicted to maintain CSF concentrations > 2.18 nM for at least 24 h.

3.4 Tyrosine Kinase Inhibitors

Tyrosine kinase inhibitors (TKI) are a class of drugs targeting specific enzymes that affect cell signal transduction pathways controlling cell proliferation, differentiation, and anti-apoptotic activity in cancer cells [67]. Administered orally, a number of these drugs have been reported to penetrate the human BBB despite conflicting preclinical studies that may suggest otherwise [68]. Their use in the treatment of primary brain tumors and brain metastases has been evaluated over the past 10 years.

Gefitinib, a small molecule TKI that reversibly inhibits epidermal growth factor receptor (EGFR), is marketed in over 64 countries outside the United States for treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC) with sensitizing EGFR mutations [69–71]. Gefitinib has a molecular weight of 446 g/mol, is highly protein bound (~97%), is a P-gp and BCRP substrate, and is distributed widely throughout the body after administration [72].

Hofer and Frei obtained glioblastoma tissue and plasma samples from seven patients receiving 500 mg/day gefitinib [73]. The average (range) concentrations for tumor tissue and plasma were 7,694 ng/g (2852 – 24103 ng/g) and 339.7 nM (8–553 nM), respectively with a median tumor/plasma ratio of 37. However, it's difficult to interpret these results because one patient has three tumor tissue samples with three-fold variability and no plasma sample with which to compare. Moreover, high interpatient variability was observed in tumor penetration, which could be attributed to tumor heterogeneity, the degree of tumor vasculature in the sample, or the fact that some patients may not have achieved steady state, since tumor and plasma samples were obtained as early as 5 days into treatment.

Zhao et al. obtained both plasma and CSF LP samples 4h after administration of a standard dose (250 mg/day) of gefitinib on day 7 from 22 patients with lung adenocarcinoma (LA) with suspected or confirmed CNS metastases [74]. Plasma and CSF concentrations were 1100 ± 412 nM and 13.9 ± 10.3 nM, respectively, with an average CSF penetration ratio of 0.013 ± 0.007 , consistent with a previously reported case study [75]. Though CSF penetration was poor, the authors found a significant correlation between the CSF and plasma gefitinib concentrations ($p=0.006$). They also found that CSF penetration was higher in patients with CNS metastases ($p=0.042$). The authors argue that actual CSF penetration ratio is closer to 0.5 considering the presumed free-drug concentration in blood (~3% of total), and recommend doubling the gefitinib dosage in order to increase CSF penetration in future studies. However, this is assuming all drug in CSF is unbound and gefitinib exhibits linear pharmacokinetics across dosages in the CSF and brain. One case study documented

increased CSF concentrations with increasing gefitinib dosage, but further studies are needed to validate this result [76].

Togashi et al. compared the CSF penetration of gefitinib and another TKI, erlotinib, in 15 Japanese patients with NSCLC or LA with CNS metastases and EGFR mutations [77]. Patients received either 250 mg daily gefitinib (n=6) or 150 mg daily erlotinib (n=7), and two patients received both drugs at different times. At steady state, total gefitinib CSF concentrations were 8.2 ± 4.3 nM with a CSF penetration ratio of 0.0113 ± 0.0036 , similar to the Zhao study [74]. Total erlotinib CSF concentrations were 66.9 ± 39 nM with a CSF penetration ratio of 0.0277 ± 0.0045 . These results might suggest that erlotinib has better CNS penetration than gefitinib, but the small number of patients studied, the wide interpatient variability, and heterogeneity in previous therapy (primarily radiation therapy) prevent one from reaching any firm conclusions.

Erlotinib is an EGFR targeting TKI that is FDA approved to treat NSCLC brain metastases with EGFR sensitizing mutations and is also approved as maintenance therapy for patients with progression free survival after first line therapy with platinum agents [78]. Due to evidence of CNS penetration, erlotinib is also under investigation for the treatment of primary brain tumors [79]. It has a molecular weight of 393 g/mol, is 92–95% protein bound in human plasma and its active metabolite, OSI-420, is a substrate for P-gp and BCRP [80, 81].

Broniscer et al. collected CSF and plasma PK samples from a pediatric patient with glioblastoma receiving oral erlotinib 78 mg/m² daily (10% lower than recommended adult dose of 150 mg/day) along with local irradiation [79]. Simultaneous serial CSF (via externalized ventriculoperitoneal shunt) and plasma samples were obtained on day 34 of therapy, and the CSF to plasma AUC ratio of erlotinib and OSI-420 was ~ 0.07 and ~0.09. Due to disruption of the BBB in high-grade gliomas, penetration into the brain may be higher, but further studies must be conducted to investigate this hypothesis.

Togashi et al. administered 150 mg daily erlotinib to 8 Asian adult patients with NSCLC with CNS metastases and measured single, simultaneous CSF and plasma erlotinib samples on day 8 [82, 83]. The mean $\pm$ SD CSF penetration ratio of erlotinib was 0.045 ± 0.015 , with an average CSF concentration of 106 ± 59 nM. The inclusion criteria permitted a wide range of disease severity with various prior therapies, which may limit replication of these results in a larger population.

Combination of erlotinib with other chemotherapeutic agents is under investigation to improve antitumor response in patients with lung adenocarcinoma (LA). Yang et al. treated 3 patients with LA and leptomeningeal metastasis (LM) with pemetrexed, cisplatin, and erlotinib and an additional 3 patients with erlotinib alone [84]. Steady state CSF and plasma samples were collected for patients receiving erlotinib only and patients receiving combination therapy. No statistical difference (p=0.44) was noted for the erlotinib CSF penetration ratio between the erlotinib only group (mean $\pm$ SD, 0.020 ± 0.005) and the combination group (0.023 ± 0.002), suggesting that adding pemetrexed/cisplatin to erlotinib therapy does not change the erlotinib CSF penetration. While the reported erlotinib CSF

penetration was similar to those mentioned previously [77, 83, 85], the small sample size necessitates further studies to substantiate these findings.

Lapatinib is FDA approved for use with capecitabine in previously treated patients with metastatic breast cancer overexpressing HER2 and is in clinical trials in combination therapies for both adult and pediatric brain tumors [78, 86]. Lapatinib has an intermediate molecular weight of 581 g/mol, is 99% protein bound, and is a substrate for P-gp and BCRP [59, 68].

In a recent study in children with CNS tumors, 900 mg/m² of lapatinib was given orally twice a day for 28 d [87]. Three patients had a single matched plasma and tumor sample collected during surgery. The median (range) total plasma and tumor concentrations in the three patients with matched samples were 3.92 µM (1.68–9.17 µM) and 516 ng/g (173 – 599 ng/g), respectively. Although this study demonstrated encouraging lapatinib tumor penetration of ~10–20%, no difference in ERBB receptor inhibition was found by western blotting likely because free lapatinib concentrations were not sufficient for significant pharmacodynamic effect.

Vivanco et al. conducted a multicenter trial enrolling 44 patients with recurrent glioblastoma receiving 750 mg/day lapatinib [88]. Surgery was performed and matched plasma and tumor samples were collected on day 7. Despite considerable interpatient variability in lapatinib plasma (324–3991 nM), and mean tumor concentrations (70–3826 nM), the results agreed with previously published results [89]. Tumor penetration ratios were only calculable in 23 patients and ranged (mean ± SD) from 0.03–2.16 (0.61±0.6). Of note, lapatinib concentrations were not corrected for protein binding or blood contained within the tumor vasculature.

Morikawa et al. studied four patients with HER2 sensitive metastatic breast cancer receiving lapatinib 1250 mg/day for 2–5 days prior to surgical resection. Tumor tissue was collected during surgery and serum was collected before, during, and after surgery [59]. Lapatinib total tumor concentrations ranged from 0.7 – 77.2 µM at two to three hours post-dose, tumor penetration ratios ranged from 0.19–9.8, and concentrations increased with number of administered lapatinib doses since patients were not at steady-state upon tumor resection. While serum concentrations were consistent between patients, high inter and intra-patient variability in lapatinib tumor concentrations was observed.

3.5 Antifolates

Antifolates are a class of medications that exert their mechanism of action through intracellular depletion of reduced folates by inhibition of dihydrofolate reductase (DHFR) or thymidylate synthetase (TS), preventing the production of purines and pyrimidines for DNA and RNA synthesis [90]. Antifolates are widely used in the treatment of many diseases including malaria, rheumatoid arthritis, psoriasis, and cancer.

Methotrexate (MTX) is FDA approved for the treatment of acute lymphoblast leukemia (ALL), non-Hodgkin's lymphoma (NHL), osteosarcoma, psoriasis, and rheumatoid arthritis and is in clinical trials for adult glioma patients (NCT00463008) as well as children with

choroid plexus carcinoma (NCT00602667) [91–96]. It is a folate analog that undergoes intracellular polyglutamylation by folypoly-gamma-glutamate synthetase (FPGS) and irreversibly binds to DHFR. MTX has a molecular weight of 454 g/mol, is moderately (50%) protein bound, is renally eliminated, and is a substrate for P-gp and BCRP [97] [98]. The pharmacokinetics and CNS penetration of MTX vary depending on the route of administration [4].

Csordas and colleagues reported data on 153 pediatric patients enrolled on two separate ALL clinical trials between 1998 and 2010 receiving 1–4 courses of either 2 or 5 g/m² high-dose methotrexate (HDMTX) over 24 h [99]. The median (interquartile range) CSF MTX concentrations 24 h after the start of infusion in patients receiving 5 and 2 g/m² were 1.7 µM (0.99 – 2.78 µM) and 0.62 µM (0.48 – 1.04 µM), respectively. The median MTX CSF penetration based on the ratio of 24 h CSF to serum MTX concentration was similar for both groups (0.025) and more than half of patients from each group achieved CSF penetration ratios between 0.01–0.03. A significant but moderate correlation (Spearman's $r \approx 0.35$) was observed between serum and CSF MTX concentrations in both groups at 24 h. It is difficult to compare these results with previous studies as details on CSF sample acquisition (collection site, sample volume) were not provided and only a single CSF sample was taken from each patient.

Jonsson and colleagues investigated the relationship between serum and CSF MTX pharmacokinetics and the risk of CNS relapse in children with ALL. This retrospective study examined 353 patients treated on two protocols who received 5 or 8 g/m² HDMTX over 24 h [100]. In a small subpopulation (34 patients) CSF MTX concentrations were collected at EOI and values ranged from 0.29 – 10.5 µM with a mean (range) CSF/plasma ratio of 0.018 (0.002 – 0.12). The investigators used this data to develop a linear mixed effects model for prediction of MTX CSF EOI concentration in the overall study population. The authors found median serum MTX concentration and number of courses with CSF concentrations > 1 µM to be associated with decreased risk of CNS relapse in different risk groups. However, this approach may not be applicable across patient populations due to the high inter-patient variability in MTX pharmacokinetics and should be validated in a larger patient population. Additionally, the use of two different assays to measure MTX concentrations along with a lack of details regarding how CSF was obtained limit the interpretation of these results.

Blakely and colleagues from the New Approaches to Brain Tumor Therapy consortium performed a clinical microdialysis MTX PK study in four patients with recurrent high grade glioma to determine if therapeutic exposures were achieved in the tumor [101]. Microdialysis catheters were placed into brain tissue adjacent to the resection cavity or through the biopsy burr hole into the tumor 1 day prior to MTX administration (12 g/m² IV over 4 h). Cerebral drug penetration, defined by the area under the MTX concentration-time curve in brain ECF compared to plasma, was found to be greater in contrast enhancing tumors (0.28–0.31) than non-enhancing tumors (0.032–0.094) suggesting greater BBB disruption and drug exchange at contrast enhancing sites.

Pemetrexed (PMX) is a multi-targeted antifolate that is FDA-approved in adults for the treatment of nonsquamous, non-small cell lung cancer (NSCLC) and mesothelioma, and is in a clinical trial with gemcitabine for pediatric medulloblastoma patients (NCT01878617) [102, 103]. It undergoes rapid polyglutamylation with greater potency (K_m) for mammalian FPGS than MTX (0.8 μ M vs. 166 μ M) [104]. PMX has a molecular weight of 427 g/mol, is ~80% protein bound, is renally eliminated, and is a substrate for P-gp and BCRP with limited tissue distribution [105, 106].

Kumthekar and colleagues reported on 21 patients with brain or leptomeningeal metastases on two different protocols to investigate PMX CSF penetration and anti-tumor activity [107]. Patients received PMX IV over 10 minutes every 3 weeks and 18 patients enrolled on the PK study had a single paired CSF and plasma sample collected after the first dose while three patients with an Ommaya reservoir underwent serial CSF and plasma sampling. The authors report a median CSF to plasma AUC ratio of 0.02 – 0.03. A limitation of the study design is the PMX CSF C_{max} is delayed compared to plasma C_{max} and was likely not captured in patients that provided a single time point 30–60 post infusion. In the 3 patients that underwent serial CSF/plasma sampling, the CSF C_{max} occurred at 1, 4, and 6 h. The authors showed limited PMX CSF penetration in this study, however additional studies are needed to accurately describe the CNS disposition and determine if a dosage-penetration relationship exists.

3.6 Antibodies

Monoclonal antibodies are large, hydrophilic macromolecules that are often not considered in the treatment of CNS disease due to their perceived inability to penetrate the blood brain barrier at concentrations necessary for anti-tumor efficacy. Although one approach for monoclonal antibody penetration through the BBB is by receptor-mediated transcytosis systems present on brain endothelial cells, which allows transport of macromolecules including proteins, this has not proved successful to date for anticancer drug to treat CNS tumors [3, 108]. Currently, when treating CNS metastases, these agents may be combined with radiotherapy in an attempt to alter the integrity of the BBB and improve delivery of monoclonal antibodies.

Rituximab is an anti-CD20 monoclonal antibody that is FDA approved for the treatment of CD20 positive non-Hodgkins lymphoma (NHL), CD20 chronic lymphocytic leukemia (CLL), and in combination with oral methotrexate for moderate to severe rheumatoid arthritis [109–111]. Multiple case reports have been published describing rituximab CSF concentrations after IV or intraventricular administration [112–114]. The limited data on rituximab CNS pharmacokinetics from case reports show that it has some CSF penetration but exhibits extensive interpatient variability in penetration (e.g., 0.0016 to 0.176).

Petereit and Rubbert-Roth investigated the use of 4 weekly rituximab infusions (375 mg/m²) in three adults with neurological autoimmune disorders, two with multiple sclerosis (MS) [112]. A total of 14 paired serum and CSF samples were obtained pre and post treatment, but only half of the CSF samples were quantifiable and one patient did not have measurable CSF concentrations. In the MS patients, the median (range) CSF penetration at various time points is 0.0016 (0.0004 – 0.0024) and the highest CSF concentrations were 170 and 201

ng/mL, measured at weeks 3 and 4 respectively. Although the assay is not highly specific, the authors comment that depletion of CSF B-cells is evidence of rituximab activity and its ability to penetrate the BCSFB. Ruhstaller and colleagues describe a case report of IV rituximab (800 mg/week) given to a 38 year old patient with NHL and CNS involvement [113]. After four weekly infusions, the patient received alternating weeks of intrathecal therapy and IV rituximab for a total of 12 rituximab IV infusions and 5 intrathecal administrations. Rituximab was measured in the CSF and serum 4 days after the 6th infusion and right after the 7th infusion and demonstrated a CSF to serum ratio of 0.17 and 0.01, respectively. Harjunpaa and colleagues evaluated the use of 4 weekly rituximab 375 mg/m² infusions in a 57 y/o NHL patient with CNS involvement. Serial CSF and blood samples were collected with the first infusion and peak or trough samples with the other infusions [115]. CSF rituximab concentrations increased with number of infusions but never exceeded 0.55 µg/mL with a CSF penetration of 0.002. Without details on CSF sample collection it is unknown if there are differences between ventricular and lumbar concentrations of rituximab.

3.7 Smoothened Inhibitors

The hedgehog (Hh) signaling pathway controls cell growth and differentiation during embryonic development [116]. In the majority of adults, this pathway is inactive, but has been implicated in the pathogenesis of two malignancies, basal cell carcinoma and medulloblastoma [117, 118]. Activation of the Hh pathway can cause tumorigenesis through mutations in PTCH1, smoothened (SMO), or through production of Hh ligand causing inhibition of PTCH1.

Vismodegib (GDC-0449) is a small molecule Hh pathway SMO inhibitor that prevents downstream activation of Hh target genes. It received FDA approval in early 2012 for the treatment of adults with metastatic basal cell carcinoma or locally advanced basal cell carcinoma that has recurred after surgery or cannot be treated with surgery or radiation [119]. It is also undergoing clinical trials for numerous indications including medulloblastoma (NCT01239316) and chondrosarcoma (NCT01267955). Vismodegib has a molecular weight of 421 g/mol, is highly (99%) protein bound, and has not been shown to be a substrate for efflux transporters in the brain.

In a study of 33 pediatric patients with recurrent and refractory medulloblastoma, Gajjar et al. provided a summary of vismodegib total and unbound plasma pharmacokinetic parameters [120]. A total of three patients with ventricular access devices consented to provide simultaneous ventricular CSF and plasma samples at 1, 3, and 8 h after the vismodegib dose. The ratio of vismodegib AUC₀₋₈ in CSF to plasma (total or unbound) was used as a measure of CSF drug penetration. The median (range) vismodegib drug penetration in CSF compared to total vismodegib in plasma was 0.0026 (0.0014–0.0062) and compared to unbound vismodegib in plasma was 0.53 (0.26–0.78). The interpretation of these results is complicated somewhat by the recent findings of Nguyen and colleagues with etravirin [121], a drug highly bound to CSF proteins. These results show that CSF drug disposition could be affected by protein binding in the CSF, which would lead to the

question of how different unbound vismodegib CSF disposition would be from total CSF drug disposition.

4. Discussion

In patients with primary or metastatic CNS tumors, the use of anticancer agents often does not result in an increase in overall survival. As reviewed above, few anticancer drugs can penetrate into brain and tumor tissue at sufficient concentrations for adequate duration (i.e., drug exposure) to cause a significant antitumor effect. It is assumed low unbound drug concentrations in the tumor will result in a lack of target site inhibition or cytotoxicity but rarely are pharmacodynamic studies conducted to confirm this hypothesis. It will be necessary for future studies to incorporate validated pharmacodynamics measures (e.g., biomarkers) to establish drug success or failure through the development of PK-PD-efficacy relationships.

Many clinical CNS PK studies aim to collect both plasma and CNS tissue/CSF samples but are limited due to the number of patients enrolled and number of samples collected (often just one). A large variability is observed in the results because samples are collected at differing time points and occasionally sample collection time is not provided. Since multiple methods are used to determine CNS penetration in the clinical setting, comparisons among studies remain difficult. The use of CSF drug concentrations as a surrogate for brain and/or tumor penetration is misleading due to differences in the brain, CSF, and tumor tissue barriers. In this manuscript we have highlighted limitations to the different methodologies used to assess the penetration of anticancer agents into the CNS, but due to the complexity of the different barriers, tumor types, and drugs used we cannot provide a recommendation on the “best” methods to assess CNS drug penetration. However, we point the reader to several recently published articles focusing on techniques to determine CNS drug penetration [6, 122–125]. Although each reported technique has different strengths and limitations, CSF sampling remains the most accessible method to estimate unbound drug concentrations in the tumor ECF.[122].

In conclusion, several issues should be considered when assessing the CNS exposure of drugs currently in clinical development or already in the clinic, or new chemical entities as they are evaluated in upcoming clinical trials. Since most drugs used to treat CNS tumors were initially identified to treat peripheral malignancies (most often leukemia or lymphoma where the drug target is located in plasma), these agents were specifically designed to not penetrate into the brain to avoid CNS side effects. There is a growing need for anticancer therapies in the treatment of CNS tumors as more effective systemic therapy has improved survival but is resulting in a growing incidence of brain metastases [126]. More recently chemists have begun to design and develop analogs that are penetrant to treat CNS tumors [127–129]. With a renewed focus in drug development for treating CNS tumors, it will be crucial that precise and accurate quantitation of CNS exposures be determined so that systemic dosing can be optimized to improve the likelihood of increasing the survival in patients with CNS tumors.

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References

1. Ostrom QT, Gittleman H, Liao P, Rouse C, Chen Y, Dowling J, et al. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2007–2011. 2014 Oct 01.
2. Bertolini F, Spallanzani A, Fontana A, Depenni R, Luppi G. Brain metastases: an overview. *CNS Oncol.* 2015; 4(1):37–46. [PubMed: 25586424]
3. Chen Y, Liu L. Modern methods for delivery of drugs across the blood-brain barrier. *Adv Drug Deliv Rev.* 2012 May; 64(7):640–65. [PubMed: 22154620]
4. Motl S, Zhuang Y, Waters CM, Stewart CF. Pharmacokinetic considerations in the treatment of CNS tumours. *Clin Pharmacokinet.* 2006; 45(9):871–903. [PubMed: 16928151]
5. Abbott NJ, Romero IA. Transporting therapeutics across the blood-brain barrier. *Mol Med Today.* 1996 Mar; 2(3):106–13. [PubMed: 8796867]
6. Abbott NJ. Blood-brain barrier structure and function and the challenges for CNS drug delivery. *J Inher Metab Dis.* 2013 May; 36(3):437–49. [PubMed: 23609350]
7. Cardoso FL, Brites D, Brito MA. Looking at the blood-brain barrier: molecular anatomy and possible investigation approaches. *Brain Res Rev.* 2010 Sep; 64(2):328–63. [PubMed: 20685221]
8. Deeken JF, Löscher W. The blood-brain barrier and cancer: transporters, treatment, and Trojan horses. *Clin Cancer Res.* 2007 Mar; 13(6):1663–74. [PubMed: 17363519]
9. Pardridge WM. The blood-brain barrier: bottleneck in brain drug development. *NeuroRx.* 2005 Jan; 2(1):3–14. [PubMed: 15717053]
10. Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. *NeuroRx.* 2005 Oct; 2(4):541–53. [PubMed: 16489364]
11. Pardridge WM. Drug transport across the blood-brain barrier. *J Cereb Blood Flow Metab.* 2012 Nov; 32(11):1959–72. [PubMed: 22929442]
12. Nau R, Sörgel F, Eiffert H. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. *Clin Microbiol Rev.* 2010 Oct; 23(4): 858–83. [PubMed: 20930076]
13. Hammarlund-Udenaes M, Friden M, Syvanen S, Gupta A. On the rate and extent of drug delivery to the brain. *Pharm Res.* 2008 Aug; 25(8):1737–50. [PubMed: 18058202]
14. de Lange EC. Utility of CSF in translational neuroscience. *J Pharmacokinet Pharmacodyn.* 2013 Jun; 40(3):315–26. [PubMed: 23400635]
15. Rambeck B, Jürgens UH, May TW, Pannek HW, Behne F, Ebner A, et al. Comparison of brain extracellular fluid, brain tissue, cerebrospinal fluid, and serum concentrations of antiepileptic drugs measured intraoperatively in patients with intractable epilepsy. *Epilepsia.* 2006 Apr; 47(4): 681–94. [PubMed: 16650134]
16. Abbott NJ, Romero IA. Transporting therapeutics across the blood-brain barrier. *Molecular Medicine Today.* 1996; 2(3):106–13. [PubMed: 8796867]
17. Pardridge WM. Blood-brain barrier drug targeting: the future of brain drug development. *Mol Interv.* 2003 Mar; 3(2):90–105. 51. [PubMed: 14993430]
18. Pardridge WM, Boado RJ, Black KL, Cancilla PA. Blood-brain barrier and new approaches to brain drug delivery. *West J Med.* 1992 Mar; 156(3):281–6. [PubMed: 1595245]
19. Pokorny JL, Calligaris D, Gupta SK, Iyekege DO, Mueller D, Bakken KK, et al. 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.* 2015 Apr; 21(8): 1916–24. [PubMed: 25609063]

20. On NH, Mitchell R, Savant SD, Bachmeier CJ, Hatch GM, Miller DW. Examination of blood-brain barrier (BBB) integrity in a mouse brain tumor model. *J Neurooncol.* 2013 Jan; 111(2):133–43. [PubMed: 23184143]
21. Groothuis DR. The blood-brain and blood-tumor barriers: a review of strategies for increasing drug delivery. *Neuro Oncol.* 2000 Jan; 2(1):45–59. [PubMed: 11302254]
22. van Tellingen O, Yetkin-Arik B, de Gooijer MC, Wesseling P, Wurdinger T, de Vries HE. Overcoming the blood-brain tumor barrier for effective glioblastoma treatment. *Drug Resist Updat.* 2015 Mar; 19:1–12. [PubMed: 25791797]
23. Kim JJ, Tannock IF. Repopulation of cancer cells during therapy: an important cause of treatment failure. *Nat Rev Cancer.* 2005 Jul; 5(7):516–25. [PubMed: 15965493]
24. Lockman PR, Mittapalli RK, Taskar KS, Rudraraju V, Gril B, Bohn KA, et al. Heterogeneous blood-tumor barrier permeability determines drug efficacy in experimental brain metastases of breast cancer. *Clin Cancer Res.* 2010 Dec; 16(23):5664–78. [PubMed: 20829328]
25. On NH, Miller DW. Transporter-based delivery of anticancer drugs to the brain: improving brain penetration by minimizing drug efflux at the blood-brain barrier. *Curr Pharm Des Netherlands.* 2014:1499–509.
26. Stieger B, Gao B. Drug transporters in the central nervous system. *Clin Pharmacokinet.* 2015 Mar; 54(3):225–42. [PubMed: 25647629]
27. Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, et al. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell.* 1994; 77(4):491–502. [PubMed: 7910522]
28. Rao VV, Dahlheimer JL, Bardgett ME, Snyder AZ, Finch RA, Sartorelli AC, et al. Choroid plexus epithelial expression of MDR1 P glycoprotein and multidrug resistance-associated protein contribute to the blood-cerebrospinal-fluid drug-permeability barrier. *Proc Natl Acad Sci USA.* 1999; 96(7):3900–5.
29. Loscher W, Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. *Nat Rev Neurosci.* 2005 Aug; 6(8):591–602. [PubMed: 16025095]
30. Ginguene C, Champier J, Maallem S, Strazielle N, Jouvét A, Fevre-Montange M, et al. P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) localize in the microvessels forming the blood-tumor barrier in ependymomas. *Brain Pathol Switzerland.* 2010:926–35.
31. Callaghan, R.; Luk, F.; Bebawy, M. *Drug Metab Dispos.* United States: 2014. Inhibition of the multidrug resistance P-glycoprotein: time for a change of strategy?; p. 623-31.
32. Damia G, D'Incalci M. Mechanisms of resistance to alkylating agents. *Cytotechnology.* 1998:165–73. [PubMed: 19002790]
33. Patel M, McCully C, Godwin K, Balis FM. Plasma and cerebrospinal fluid pharmacokinetics of intravenous temozolomide in non-human primates. *J Neurooncol.* 2003 Feb; 61(3):203–7. [PubMed: 12675312]
34. Goldwirt L, Beccaria K, Carpentier A, Farinotti R, Fernandez C. Irinotecan and temozolomide brain distribution: a focus on ABCB1. *Cancer Chemother Pharmacol.* 2014 Jul; 74(1):185–93. [PubMed: 24867782]
35. Baker SD, Wirth M, Statkevich P, Reidenberg P, Alton K, Sartorius SE, et al. Absorption, metabolism, and excretion of 14 C-temozolomide following oral administration to patients with advanced cancer. *Clinical Cancer Research.* 1999; 5:309–17. [PubMed: 10037179]
36. Riganti C, Salaroglio IC, Pinzon-Daza ML, Caldera V, Campia I, Kopecka J, et al. Temozolomide down-regulates P-glycoprotein in human blood-brain barrier cells by disrupting Wnt3 signaling. *Cell Mol Life Sci.* 2014 Feb; 71(3):499–516. [PubMed: 23771630]
37. Portnow J, Badie B, Chen M, Liu A, Blanchard S, Synold TW. The neuropharmacokinetics of temozolomide in patients with resectable brain tumors: potential implications for the current approach to chemoradiation. *Clin Cancer Res.* 2009 Nov 15; 15(22):7092–8. [PubMed: 19861433]
38. Zhou Q, Guo P, Kruh GD, Vicini P, Wang X, Gallo JM. Predicting human tumor drug concentrations from a preclinical pharmacokinetic model of temozolomide brain disposition. *Clin Cancer Res.* 2007 Jul 15; 13(14):4271–9. [PubMed: 17634557]

39. Ostermann S, Csajka C, Buclin T, Leyvraz S, Lejeune F, Decosterd LA, et al. Plasma and cerebrospinal fluid population pharmacokinetics of temozolomide in malignant glioma patients. *Clin Cancer Res*. 2004 Jun 1; 10(11):3728–36. [PubMed: 15173079]
40. Rosso L, Brock CS, Gallo JM, Saleem A, Price PM, Turkheimer FE, et al. A new model for prediction of drug distribution in tumor and normal tissues: pharmacokinetics of temozolomide in glioma patients. *Cancer Res*. 2009 Jan 1; 69(1):120–7. [PubMed: 19117994]
41. Rodeghiero F, Elice F. Ifosfamide in hematological malignancies of adults. *Oncology*. 2003; 65(Suppl 2):85–93. [PubMed: 14586156]
42. Di Cataldo A, Astuto M, Rizzo G, Bertuna G, Russo G, Incorpora G. Neurotoxicity during ifosfamide treatment in children. *Med Sci Monit*. 2009 Jan; 15(1):Cs22–5. [PubMed: 19114973]
43. Goldberg RM, Erlichman C. Irinotecan plus 5-FU and leucovorin in advanced colorectal cancer: North American trials. *Oncology (Huntingt)*. 1998; 12(8 Suppl 6):59–63. [PubMed: 9726093]
44. Lokiec F. Ifosfamide: pharmacokinetic properties for central nervous system metastasis prevention. *Ann Oncol*. 2006 May; 17(Suppl 4):iv33–6. [PubMed: 16702183]
45. Kiewe P, Neumann M, Wagner T, Seyfert S, Albrecht H, Thiel E, et al. Penetration of ifosfamide and its active metabolite 4-OH-ifosfamide into cerebrospinal fluid of patients with CNS malignancies. *Cancer Chemother Pharmacol*. 2011 Jan; 67(1):27–33. [PubMed: 20182727]
46. Creaven PJ, Allen LM, Alford DA, Cohen MH. Clinical pharmacology of isophosphamide. *Clin Pharmacol Ther*. 1974 Jul; 16(1):77–86. [PubMed: 4602057]
47. Kaijser GP, De Kraker J, Bult A, Underberg WJ, Beijnen JH. Pharmacokinetics of ifosfamide and some metabolites in children. *Anticancer Research*. 1998; 18(3B):1941–9. [PubMed: 9677448]
48. Yule SM, Price L, Pearson ADJ, Boddy AV. Cyclophosphamide and ifosfamide metabolites in the cerebrospinal fluid of children. *Clinical Cancer Research*. 1997; 3:1985–92. [PubMed: 9815588]
49. Ninane J, Baurain R, de Kraker J, Ferster A, Trouet A, Cornu G. Alkylating activity in serum, urine, and CSF following high-dose ifosfamide in children. *Cancer Chemother Pharmacol*. 1989; 24(Suppl 1):S2–6. discussion S7. [PubMed: 2503256]
50. Bartsch R, Wenzel C, Gampenrieder SP, Pluschnig U, Altorjai G, Rudas M, et al. Trastuzumab and gemcitabine as salvage therapy in heavily pre-treated patients with metastatic breast cancer. *Cancer Chemother Pharmacol*. 2008 Oct; 62(5):903–10. [PubMed: 18256835]
51. Donadio M, Ardine M, Berruti A, Ritorto G, Fea E, Mistrangelo M, et al. Gemcitabine and vinorelbine as second-line treatment in patients with metastatic breast cancer: a phase II study. *Cancer Chemother Pharmacol*. 2003 Aug; 52(2):147–52. [PubMed: 12764672]
52. Melichar B, Urmanska H, Kohlova T, Nova M, Cesak T. Brain metastases of epithelial ovarian carcinoma responding to cisplatin and gemcitabine combination chemotherapy: a case report and review of the literature. *Gynecol Oncol*. 2004 Aug; 94(2):267–76. [PubMed: 15297161]
53. Mini E, Nobili S, Caciagli B, Landini I, Mazzei T. Cellular pharmacology of gemcitabine. *Ann Oncol*. 2006 May; 17(Suppl 5):v7–12. [PubMed: 16807468]
54. Bernardi RJ, Bomgaars L, Fox E, Balis FM, Egorin MJ, Lagattuta TF, et al. Phase I clinical trial of intrathecal gemcitabine in patients with neoplastic meningitis. *Cancer Chemother Pharmacol*. 2008 Jul; 62(2):355–61. [PubMed: 17909804]
55. Egorin MJ, Zuhowski EG, McCully CM, Blaney SM, Kerr JZ, Berg SL, et al. Pharmacokinetics of intrathecal gemcitabine in nonhuman primates. *Clin Cancer Res*. 2002 Jul; 8(7):2437–42. [PubMed: 12114450]
56. Sigmond J, Honeywell RJ, Postma TJ, Dirven CM, de Lange SM, van der Born K, et al. Gemcitabine uptake in glioblastoma multiforme: potential as a radiosensitizer. *Ann Oncol*. 2009 Jan; 20(1):182–7. [PubMed: 18701427]
57. Xeloda [prescribing information]. Genentech USA, Inc; South San Francisco, CA, USA:
58. Zhang J, Zhang L, Yan Y, Li S, Xie L, Zhong W, et al. Are capecitabine and the active metabolite 5-Fu CNS penetrable to treat breast cancer brain metastasis? *Drug Metab Dispos*. 2015; 43(3):411–7. [PubMed: 25547867]
59. Morikawa A, Peereboom DM, Thorsheim HR, Samala R, Balyan R, Murphy CG, et al. Capecitabine and lapatinib uptake in surgically resected brain metastases from metastatic breast cancer patients: a prospective study. *Neuro Oncol*. 2015 Feb; 17(2):289–95. [PubMed: 25015089]

60. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. *NatRevCancer*. 2006; 6(10): 789–802.
61. Burke, TG.; Mi, Z. *Anal Biochem*. United States: 1993. Preferential binding of the carboxylate form of camptothecin by human serum albumin; p. 285-7.
62. Stewart L, Redinbo MR, Qiu X, Hol WG, Champoux JJ. A model for the mechanism of human topoisomerase I. *Science*. 1998; 279(5356):1534–41. [PubMed: 9488652]
63. Hendricks CB, Rowinsky EK, Grochow LB, Donehower RC, Kaufmann SH. Effect of P-glycoprotein expression on the accumulation and cytotoxicity of topotecan (SK&F 104864), a new camptothecin analogue. *Cancer Res*. 1992; 52:2268–78. [PubMed: 1348448]
64. Baker SD, Heideman RL, Crom WR, Kuttlesch JF, Gajjar A, Stewart CF. Cerebrospinal fluid pharmacokinetics and penetration of continuous infusion topotecan in children with central nervous system tumors. *Cancer ChemotherPharmacol*. 1996; 37(3):195–202.
65. Morgan RJ, Synold T, Mamelak A, Lim D, Al-Kadhimi Z, Twardowski P, et al. Plasma and cerebrospinal fluid pharmacokinetics of topotecan in a phase I trial of topotecan, tamoxifen, and carboplatin, in the treatment of recurrent or refractory brain or spinal cord tumors. *Cancer Chemother Pharmacol*. 2010 Oct; 66(5):927–33. [PubMed: 20107803]
66. Blaney SM, Tagen M, Onar-Thomas A, Berg SL, Gururangan S, Scorsone K, et al. A phase-1 pharmacokinetic optimal dosing study of intraventricular topotecan for children with neoplastic meningitis: a Pediatric Brain Tumor Consortium study. *Pediatr Blood Cancer*. 2013 Apr; 60(4): 627–32. [PubMed: 23002039]
67. Gross S, Rahal R, Stransky N, Lengauer C, Hoeflich KP. Targeting cancer with kinase inhibitors. *J Clin Invest*. 2015 May 1; 125(5):1780–9. [PubMed: 25932675]
68. Scheffler M, Di Gion P, Doroshyenko O, Wolf J, Fuhr U. Clinical pharmacokinetics of tyrosine kinase inhibitors: focus on 4-anilinoquinazolines. *Clin Pharmacokinet*. 2011 Jun; 50(6):371–403. [PubMed: 21553932]
69. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*. 2009 Sep; 361(10):947–57. [PubMed: 19692680]
70. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol*. 2010 Feb; 11(2):121–8. [PubMed: 20022809]
71. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med*. 2010 Jun; 362(25):2380–8. [PubMed: 20573926]
72. Agarwal, S.; Sane, R.; Gallardo, JL.; Ohlfest, JR.; Elmquist, WF. *J Pharmacol Exp Ther*. United States: 2010. Distribution of gefitinib to the brain is limited by P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2)-mediated active efflux; p. 147-55.
73. Hofer S, Frei K. Gefitinib concentrations in human glioblastoma tissue. *J Neurooncol*. 2007 Apr; 82(2):175–6. [PubMed: 17008949]
74. Zhao J, Chen M, Zhong W, Zhang L, Li L, Xiao Y, et al. Cerebrospinal fluid concentrations of gefitinib in patients with lung adenocarcinoma. *Clin Lung Cancer*. 2013 Mar; 14(2):188–93. [PubMed: 22846582]
75. Fukuhara T, Saijo Y, Sakakibara T, Inoue A, Morikawa N, Kanamori M, et al. Successful treatment of carcinomatous meningitis with gefitinib in a patient with lung adenocarcinoma harboring a mutated EGF receptor gene. *Tohoku J Exp Med*. 2008 Apr; 214(4):359–63. [PubMed: 18441512]
76. Jackman DM, Holmes AJ, Lindeman N, Wen PY, Kesari S, Borrás AM, et al. Response and resistance in a non-small-cell lung cancer patient with an epidermal growth factor receptor mutation and leptomeningeal metastases treated with high-dose gefitinib. *J Clin Oncol*. 2006 Sep; 24(27):4517–20. [PubMed: 16983123]
77. Togashi Y, Masago K, Masuda S, Mizuno T, Fukudo M, Ikemi Y, et al. Cerebrospinal fluid concentration of gefitinib and erlotinib in patients with non-small cell lung cancer. *Cancer Chemother Pharmacol*. 2012 Sep; 70(3):399–405. [PubMed: 22806307]

78. Lin NU. Targeted therapies in brain metastases. *Curr Treat Options Neurol*. 2014 Jan;16(1):276. [PubMed: 24353011]
79. Broniscer A, Panetta JC, O'Shaughnessy M, Fraga C, Bai F, Krasin MJ, et al. Plasma and cerebrospinal fluid pharmacokinetics of erlotinib and its active metabolite OSI-420. *Clin Cancer Res*. 2007; 13(5):1511–5. [PubMed: 17332296]
80. Jamal-Hanjani M, Spicer J. Epidermal growth factor receptor tyrosine kinase inhibitors in the treatment of epidermal growth factor receptor-mutant non-small cell lung cancer metastatic to the brain. *Clin Cancer Res*. 2012 Feb 15; 18(4):938–44. [PubMed: 22167408]
81. Elmeliegy, MA.; Carcaboso, AM.; Tagen, M.; Bai, F.; Stewart, CF. *Clin Cancer Res*. United States: 2011. Role of ATP-binding cassette and solute carrier transporters in erlotinib CNS penetration and intracellular accumulation; p. 89-99.2010 Aacr
82. Togashi Y, Masago K, Fukudo M, Terada T, Fujita S, Irisa K, et al. Cerebrospinal fluid concentration of erlotinib and its active metabolite OSI-420 in patients with central nervous system metastases of non-small cell lung cancer. *J Thorac Oncol*. 2010 Jul; 5(7):950–5. [PubMed: 20479691]
83. Togashi Y, Masago K, Fukudo M, Tsuchido Y, Okuda C, Kim YH, et al. Efficacy of increased-dose erlotinib for central nervous system metastases in non-small cell lung cancer patients with epidermal growth factor receptor mutation. *Cancer Chemother Pharmacol*. 2011 Oct; 68(4):1089–92. [PubMed: 21681573]
84. Yang H, Yang X, Zhang Y, Liu X, Deng Q, Zhao M, et al. Erlotinib in combination with pemetrexed/cisplatin for leptomeningeal metastases and cerebrospinal fluid drug concentrations in lung adenocarcinoma patients after gefitinib failure. *Target Oncol*. 2014 Jul 2.
85. Clarke JL, Pao W, Wu N, Miller VA, Lassman AB. High dose weekly erlotinib achieves therapeutic concentrations in CSF and is effective in leptomeningeal metastases from epidermal growth factor receptor mutant lung cancer. *J Neurooncol*. 2010 Sep; 99(2):283–6. [PubMed: 20146086]
86. Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med*. 2006 Dec; 355(26):2733–43. [PubMed: 17192538]
87. Fouladi M, Stewart CF, Blaney SM, Onar-Thomas A, Schaiquevich P, Packer RJ, et al. A molecular biology and phase II trial of lapatinib in children with refractory CNS malignancies: a pediatric brain tumor consortium study. *J Neurooncol*. 2013 Sep; 114(2):173–9. [PubMed: 23836190]
88. Vivanco I, Robins HI, Rohle D, Campos C, Grommes C, Nghiemphu PL, et al. Differential sensitivity of glioma- versus lung cancer-specific EGFR mutations to EGFR kinase inhibitors. *Cancer Discov*. 2012 May; 2(5):458–71. [PubMed: 22588883]
89. Burris HA 3rd, Taylor CW, Jones SF, Koch KM, Versola MJ, Arya N, et al. A phase I and pharmacokinetic study of oral lapatinib administered once or twice daily in patients with solid malignancies. *Clin Cancer Res*. 2009; 15(21):6702–8. [PubMed: 19825948]
90. McGuire JJ. Anticancer antifolates: current status and future directions. *Curr Pharm Des*. 2003; 9(31):2593–613. [PubMed: 14529544]
91. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med*. 2006 Jan; 354(2):166–78. [PubMed: 16407512]
92. Pui CH, Carroll WL, Meshinchi S, Arceci RJ. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. *J Clin Oncol*. 2011 Feb; 29(5):551–65. [PubMed: 21220611]
93. Patte C, Auperin A, Gerrard M, Michon J, Pinkerton R, Sposto R, et al. Results of the randomized international FAB/LMB96 trial for intermediate risk B-cell non-Hodgkin lymphoma in children and adolescents: it is possible to reduce treatment for the early responding patients. *Blood*. 2007 Apr; 109(7):2773–80. [PubMed: 17132719]
94. Jaffe N, Gorlick R. High-dose methotrexate in osteosarcoma: let the questions surcease--time for final acceptance. *J Clin Oncol*. 2008 Sep; 26(27):4365–6. [PubMed: 18802144]
95. Menter A, Gottlieb A, Feldman SR, Van Voorhees AS, Leonardi CL, Gordon KB, et al. Guidelines of care for the management of psoriasis and psoriatic arthritis: Section 1. Overview of psoriasis

- and guidelines of care for the treatment of psoriasis with biologics. *J Am Acad Dermatol*. 2008 May; 58(5):826–50. [PubMed: 18423260]
96. Choi HK, Hernán MA, Seeger JD, Robins JM, Wolfe F. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. *Lancet*. 2002 Apr; 359(9313):1173–7. [PubMed: 11955534]
 97. Walling J. From methotrexate to pemetrexed and beyond. A review of the pharmacodynamic and clinical properties of antifolates. *Invest New Drugs*. 2006 Jan; 24(1):37–77. [PubMed: 16380836]
 98. Mahato, YLaRI. *Pharmaceutical Perspectives of Cancer Therapeutics*. 2009
 99. Csordas K, Hegyi M, Eipel OT, Muller J, Erdelyi DJ, Kovacs GT. Comparison of pharmacokinetics and toxicity after high-dose methotrexate treatments in children with acute lymphoblastic leukemia. *Anticancer Drugs*. 2013 Feb; 24(2):189–97. [PubMed: 23187460]
 100. Jönsson P, Höglund P, Wiebe T, Schröder H, Seidel H, Skärby T. Methotrexate concentrations in cerebrospinal fluid and serum, and the risk of central nervous system relapse in children with acute lymphoblastic leukaemia. *Anticancer Drugs*. 2007 Sep; 18(8):941–8. [PubMed: 17667600]
 101. Blakeley JO, Olson J, Grossman SA, He X, Weingart J, Supko JG, et al. Effect of blood brain barrier permeability in recurrent high grade gliomas on the intratumoral pharmacokinetics of methotrexate: a microdialysis study. *J Neurooncol*. 2009 Jan; 91(1):51–8. [PubMed: 18787762]
 102. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, von Pawel J, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol*. 2004 May; 22(9):1589–97. [PubMed: 15117980]
 103. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. *J Clin Oncol*. 2003 Jul; 21(14):2636–44. [PubMed: 12860938]
 104. Habeck LL, Mendelsohn LG, Shih C, Taylor EC, Colman PD, Gossett LS, et al. Substrate specificity of mammalian folylpolyglutamate synthetase for 5,10-dideazatetrahydrofolate analogs. *Mol Pharmacol*. 1995 Aug; 48(2):326–33. [PubMed: 7651366]
 105. Hooijberg JH, de Vries NA, Kaspers GJ, Pieters R, Jansen G, Peters GJ. Multidrug resistance proteins and folate supplementation: therapeutic implications for antifolates and other classes of drugs in cancer treatment. *Cancer Chemother Pharmacol*. 2006 Jul; 58(1):1–12. [PubMed: 16362298]
 106. Ouellet D, Periclou AP, Johnson RD, Woodworth JR, Lalonde RL. Population pharmacokinetics of pemetrexed disodium (ALIMTA) in patients with cancer. *Cancer Chemother Pharmacol*. 2000; 46(3):227–34. [PubMed: 11021740]
 107. Kumthekar P, Grimm SA, Avram MJ, Kaklamani V, Helenowski I, Rademaker A, et al. Pharmacokinetics and efficacy of pemetrexed in patients with brain or leptomeningeal metastases. *J Neurooncol*. 2013 Apr; 112(2):247–55. [PubMed: 23354655]
 108. Niewoehner J, Bohrmann B, Collin L, Urich E, Sade H, Maier P, et al. Increased brain penetration and potency of a therapeutic antibody using a monovalent molecular shuttle. *Neuron*. 2014 Jan; 81(1):49–60. [PubMed: 24411731]
 109. Cohen SB, Emery P, Greenwald MW, Dougados M, Furie RA, Genovese MC, et al. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis Rheum*. 2006 Sep; 54(9):2793–806. [PubMed: 16947627]
 110. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med*. 2002 Jan; 346(4):235–42. [PubMed: 11807147]
 111. Robak T, Dmoszynska A, Solal-Céligny P, Warzocha K, Loscertales J, Catalano J, et al. Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. *J Clin Oncol*. 2010 Apr; 28(10):1756–65. [PubMed: 20194844]
 112. Petereit HF, Rubbert-Roth A. Rituximab levels in cerebrospinal fluid of patients with neurological autoimmune disorders. *Mult Scler*. 2009 Feb; 15(2):189–92. [PubMed: 18971221]

113. Ruhstaller TW, Amsler U, Cerny T. Rituximab: active treatment of central nervous system involvement by non-Hodgkin's lymphoma? *Ann Oncol.* 2000 Mar; 11(3):374–5. [PubMed: 10811510]
114. Rubenstein JL, Li J, Chen L, Advani R, Drappatz J, Gerstner E, et al. Multicenter phase 1 trial of intraventricular immunochemotherapy in recurrent CNS lymphoma. *Blood.* 2013; 121(5):745–51. [PubMed: 23197589]
115. Harjunpää A, Wiklund T, Collan J, Janes R, Rosenberg J, Lee D, et al. Complement activation in circulation and central nervous system after rituximab (anti-CD20) treatment of B-cell lymphoma. *Leuk Lymphoma.* 2001 Aug; 42(4):731–8. [PubMed: 11697503]
116. Ingham PW, McMahon AP. Hedgehog signaling in animal development: paradigms and principles. *Genes Dev.* 2001 Dec 1; 15(23):3059–87. [PubMed: 11731473]
117. Rubin LL, de Sauvage FJ. Targeting the Hedgehog pathway in cancer. *Nat Rev Drug Discov.* 2006 Dec; 5(12):1026–33. [PubMed: 17139287]
118. Rudin CM, Hann CL, Laterra J, Yauch RL, Callahan CA, Fu L, et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. *N Engl J Med.* 2009 Sep 17; 361(12):1173–8. [PubMed: 19726761]
119. Sekulic A, Migden MR, Oro AE, Dirix L, Lewis KD, Hainsworth JD, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. *N Engl J Med.* 2012 Jun; 366(23):2171–9. [PubMed: 22670903]
120. Gajjar A, Stewart CF, Ellison DW, Kaste S, Kun LE, Packer RJ, et al. Phase I study of vismodegib in children with recurrent or refractory medulloblastoma: a pediatric brain tumor consortium study. *Clin Cancer Res.* 2013 Nov 15; 19(22):6305–12. [PubMed: 24077351]
121. Nguyen A, Rossi S, Croteau D, Best BM, Clifford D, Collier AC, et al. Etravirine in CSF is highly protein bound. *Journal of Antimicrobial Chemotherapy.* 2013 May 01; 68(5):1161–68. [PubMed: 23335197]
122. de Lange EC, Hammarlund-Udenaes M. Translational aspects of blood-brain barrier transport and central nervous system effects of drugs: from discovery to patients. *Clin Pharmacol Ther.* 2015 Apr; 97(4):380–94. [PubMed: 25670219]
123. Bickel U. How to measure drug transport across the blood-brain barrier. *NeuroRx.* 2005 Jan; 2(1): 15–26. [PubMed: 15717054]
124. Chassidim Y, Vazana U, Prager O, Veksler R, Bar-Klein G, Schoknecht K, et al. Analyzing the blood-brain barrier: the benefits of medical imaging in research and clinical practice. *Semin Cell Dev Biol.* 2015 Feb; 38:43–52. [PubMed: 25455024]
125. Shannon RJ, Carpenter KL, Guilfoyle MR, Helmy A, Hutchinson PJ. Cerebral microdialysis in clinical studies of drugs: pharmacokinetic applications. *J Pharmacokinet Pharmacodyn.* 2013 Jun; 40(3):343–58. [PubMed: 23468415]
126. Nayak L, Lee EQ, Wen PY. Epidemiology of brain metastases. *Curr Oncol Rep.* 2012 Feb; 14(1): 48–54. [PubMed: 22012633]
127. Heffron TP, Salphati L, Alicke B, Cheong J, Dotson J, Edgar K, et al. The design and identification of brain penetrant inhibitors of phosphoinositide 3-kinase α . *J Med Chem.* 2012 Sep; 55(18):8007–20. [PubMed: 22946614]
128. Chico LK, Van Eldik LJ, Watterson DM. Targeting protein kinases in central nervous system disorders. *Nat Rev Drug Discov.* 2009 Nov; 8(11):892–909. [PubMed: 19876042]
129. Salphati L, Heffron TP, Alicke B, Nishimura M, Barck K, Carano RA, et al. Targeting the PI3K pathway in the brain—efficacy of a PI3K inhibitor optimized to cross the blood-brain barrier. *Clin Cancer Res.* 2012 Nov 15; 18(22):6239–48. [PubMed: 22992516]
130. Masuda T, Hattori N, Hamada A, Iwamoto H, Ohshimo S, Kanehara M, et al. Erlotinib efficacy and cerebrospinal fluid concentration in patients with lung adenocarcinoma developing leptomeningeal metastases during gefitinib therapy. *Cancer Chemother Pharmacol.* 2011 Jun; 67(6):1465–9. [PubMed: 21274533]
131. Gori S, Lunardi G, Inno A, Foglietta J, Cardinali B, Del Mastro L, et al. Lapatinib concentration in cerebrospinal fluid in two patients with HER2-positive metastatic breast cancer and brain metastases. *Ann Oncol.* 2014 Apr; 25(4):912–3. [PubMed: 24667724]

132. Broniscer A, Baker SD, Wetmore C, Pai Panandiker AS, Huang J, Davidoff AM, et al. Phase I trial, pharmacokinetics, and pharmacodynamics of vandetanib and dasatinib in children with newly diagnosed diffuse intrinsic pontine glioma. *Clin Cancer Res*. 2013; 19(11):3050–8. [PubMed: 23536435]
133. Porkka K, Koskenvesa P, Lundan T, Rimpilainen J, Mustjoki S, Smykla R, et al. Dasatinib crosses the blood-brain barrier and is an efficient therapy for central nervous system Philadelphia chromosome-positive leukemia. *Blood*. 2008 Aug 15; 112(4):1005–12. [PubMed: 18477770]
134. Zhou HS, Dai M, Wei Y, Wang Q, Xu N, Yin C, et al. Isolated central nervous system relapse in patient with blast-crisis chronic myeloid leukemia in durable complete cytogenetic remission on dasatinib treatment: pharmacokinetics and ABL mutation analysis in cerebrospinal fluid. *Leuk Lymphoma*. 2013 Jul; 54(7):1557–9. [PubMed: 23194002]
135. Stemmler HJ, Schmitt M, Willems A, Bernhard H, Harbeck N, Heinemann V. Ratio of trastuzumab levels in serum and cerebrospinal fluid is altered in HER2-positive breast cancer patients with brain metastases and impairment of blood-brain barrier. *Anticancer Drugs*. 2007 Jan; 18(1):23–8. [PubMed: 17159499]
136. MacDonald TJ, Vezina G, Stewart CF, Turner D, Pierson CR, Chen L, et al. Phase II study of cilengitide in the treatment of refractory or relapsed high-grade gliomas in children: A report from the Children's Oncology Group. *Neuro-oncology*. 2013; 15(10):1438–44. [PubMed: 24014381]
137. Reardon DA, Fink KL, Mikkelsen T, Cloughesy TF, O'Neill A, Plotkin S, et al. Randomized phase II study of cilengitide, an integrin-targeting arginine-glycine-aspartic acid peptide, in recurrent glioblastoma multiforme. *J Clin Oncol*. 2008; 26(34):5610–7. [PubMed: 18981465]

Key Points

1. The lack of consideration for pharmacokinetic properties of anticancer agents necessary for penetration into CNS tumors has prevented an improvement in survival for many patients with primary CNS tumors or CNS metastases.
2. The multitude of study designs and methodologies utilized to determine CNS drug penetration in the clinical setting make comparison among studies difficult and interpretation of the results potentially ambiguous.
3. A renewed focus by researchers on drug physiochemical properties in developing agents for CNS tumors is promising, but further work must be done to translate these newer therapies into improved outcomes for patients.

Table I

Reported cerebrospinal fluid (CSF) penetration of selected chemotherapeutic agents from clinical.

Drug Class/Drug	Regimen	Disease State (no. of patients)	Method of assessing CSF penetration	Results	Reference
Alkylating agents					
Temozolomide	150 mg/m ² PO	Primary/metastatic CNS tumors (7)	Median brain ECF/plasma conc	0.178 ± 0.133	[37]
Temozolomide	75–200 mg/m ² /d x 5 PO, 75–200 mg/m ² /d PO	Recurrent glioma (7)	Tissue/plasma conc	~1.3	[40]
Ifosfamide	1300–2000 mg/m ² IV	Intracerebral lymphoma (9), metastatic breast cancer (3)	Median CSF/plasma conc	0.38 (0.18–0.72; parent); 3.07 (0.62–29.12; 4-OH-IFO)	[45]
Nucleoside Analogues					
Gemcitabine	5–10 mg IVT	Neoplastic meningitis (8)	Peak CSF conc	939 ± 384 µM	[54]
Gemcitabine	500–1000 mg/mg ² IV	Recurrent glioblastoma multiforme (10)	Tumor tissue conc	0.06–3.58 nmol/g (parent); 29–72 nmol/g (dFdU)	[56]
Capecitabine	1250 mg/m ² PO	Metastatic breast cancer (8)	Median tumor/serum conc	0.28 (0.031–0.81; parent); 5.64 (1.67–12.9; 5-FU)	[59]
Camptothecins					
Topotecan	0.25–1 mg/m ² /d IV	Primary or secondary CNS malignancies (15)	CSF/plasma conc	0.218 (total topotecan)	[65]
Topotecan	0.1–0.2 mg/dose/d IVT or intralumbar	Pediatric leukemia (2), pediatric CNS tumors (16)	Peak CSF conc	16.58 µM (2.48–46.69; IVT inj); 0.12 µM (0.006–1.89; intralumbar inj)	[66]
Tyrosine Kinase Inhibitors					
Gefitinib	500 mg/d PO	Glioblastoma (7)	Median CSF/plasma conc	37.62	[73]
Gefitinib	250 mg/d PO	LA (22)	Mean CSF/plasma conc	0.013 ± 0.007	[74]
Gefitinib	250 mg/d PO	NSCLC (1), LA mets (7)	Mean CSF/plasma conc	0.0113 ± 0.004	[77]
Erlotinib	150 mg/d PO	NSCLC (1), LA (8)	Mean CSF/plasma conc	0.0277 ± 0.005	[77]
Erlotinib	78 mg/m ² /d PO	Pediatric glioblastoma (1)	CSF/plasma conc	0.069 (parent); 0.086 (OSI-420)	[79]
Erlotinib	150 mg/d PO	NSCLC (8)	Mean CSF/plasma conc	0.045 ± 0.015	[82, 83]
Erlotinib	100–150 mg/d PO	NSCLC (3)	Mean CSF/plasma conc	0.063 ± 0.061	[130]
Erlotinib	150 mg/d PO	LA (6)	CSF/plasma conc	0.02 ± 0.005 (single agent); 0.023 ± 0.002 (combination)	[84]
Lapatinib	900 mg/m ² PO BID	Pediatric CNS tumors (3)	Mean tumor/plasma conc	0.17 (0.11–0.22)	[87]
Lapatinib	750 mg/d PO	Recurrent glioblastoma (44)	Mean tumor tissue/plasma conc	0.61 ± 0.6 (0.03–2.16)	[88]

Drug Class/Drug	Regimen	Disease State (no. of patients)	Method of assessing CSF penetration	Results	Reference
Lapatinib	1250 mg/d PO	Metastatic breast cancer (4)	Unbound tumor tissue/serum conc	0.19–9.8	[59]
Lapatinib	1250 mg/d PO	Metastatic breast cancer (2)	Mean CSF/plasma conc	0.00105 (0.008–0.0013)	[131]
Vandetanib	65 mg/m ² PO	Pediatric DIPG (2)	CSF/plasma conc	0.018 (0.012–0.024)	[132]
Dasatinib	65 mg/m ² PO	Pediatric DIPG (2)	CSF/plasma conc	0.022 (0.016–0.028)	[132]
Dasatinib	140 mg/d, 60–160 mg/m ² /d PO	Adult CML (6), adult ALL (4), pediatric ALL (4)	CSF/plasma conc	0.137 (0.05–0.28)	[133]
Dasatinib	100–170 mg/d PO	CML	CSF/plasma conc	0.01–0.04	[134]
Anti-folates					
Methotrexate	2 or 5 g/m ² IV	Pediatric ALL (153)	Median CSF/serum conc	0.025 (0.017–0.03)	[99]
Methotrexate	5 or 8 g/m ² IV	Pediatric ALL (353)	Mean CSF/plasma conc	0.018 (0.002–0.12)	[100]
Methotrexate	12 g/m ² IV	Recurrent high grade glioma (4)	ECF/plasma conc	0.28, 0.31 (contrast enhanced region); 0.032, 0.094 (non-contrast enhanced region)	[101]
Pemetrexed	500–1050 mg/m ² IV	Adults with brain or leptomeningeal metastases (21)	Median CSF/plasma conc	0.0046 (0.0002–0.035)	[107]
Antibodies					
Rituximab	375 mg/m ² IV	Multiple sclerosis (2), mononeuritis multiplex (1)	Median CSF/serum conc	0.0016 (0.0004–0.0024)	[112]
Rituximab	800 mg/week IV	NHL (1)	CSF/serum conc	0.01–0.17	[113]
Rituximab	375 mg/m ² IV	NHL (1)	CSF/plasma conc	0.002	[115]
Rituximab	10, 25, 50 mg IVT	CNS lymphoma (10)	Mean peak CSF conc	194 µg/ml (10 mg); 580 µg/ml (25 mg)	[114]
Trastuzumab	Weekly: 2 mg/kg (4 mg/kg loading dose) Triweekly: 6 mg/kg (8 mg/kg loading dose)	Metastatic breast cancer (8)	CSF/serum conc	Before RT: 0.0024 Post RT: 0.013 Meningeal Carcinomatosis: 0.02	[135]
Small molecules					
Cilengitide	N/A	Pediatric high grade glioma (1)	CSF/plasma conc	0.012	[136]
Cilengitide	200 mg, 500 mg IV	Recurrent glioma (4)	CSF/plasma conc	0.002–0.128	[137]
Smoothed inhibitors					
Vismodegib	85–170 mg/m ²	Pediatric medulloblastoma (3)	Median CSF/plasma conc	0.0026 (0.0014–0.0062) (total plasma); 0.53 (0.26–0.78) (unbound plasma)	[120]

PO=oral; IV=intravenous; IVT=intraventricular; LA= lung adenocarcinoma; NSCLC=non-small cell lung carcinoma; ALL= acute lymphoblastic leukemia; CML= chronic myelogenous leukemia; DIPG= diffuse intrinsic pontine glioma; NHL= non-Hodgkin's lymphoma; CSF= cerebrospinal fluid; conc= concentration;