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Challenges of developing small-molecule kinase inhibitors for brain tumors and the need for emphasis on free drug levels

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Abstract

Despite biological rationale and significant clinical study, the pursuit of small-molecule kinase inhibitors for the treatment of brain cancers has had very limited success. This Advance-in-Brief discusses the need for drugs to achieve free brain penetration to engage their targets where CNS tumors reside. This need to achieve free, as opposed to total, drug concentrations in the brain may be a contributing factor to why so many small-molecule kinase inhibitors have not realized success in the neuro-oncology setting. For kinase targets of interest for brain cancer, either the vast majority of small-molecule inhibitors have data suggesting that free brain penetration would be limited or there are inadequate data to suggest that free brain penetration could be expected. Therefore, kinase targets of interest in the treatment of brain cancers may be inadequately assessed due to a lack of freely brain-penetrant inhibitors available for clinical study. Encouraging recent drug discovery efforts that focused on achieving free brain penetration for cancers in the CNS are highlighted. Still, further efforts are needed to enable thorough clinical evaluation of biological hypotheses.

Key words

free brain penetration | free drug hypothesis | small molecule kinase inhibitors

For those conducting drug discovery research, the pages of this journal are a continuous reminder of the need for new treatment options for patients with brain cancers. From aggressive primary brain tumors, like glioblastoma multiforme (>12 000 new diagnoses annually in the US), to the more than 150 000 cases of brain metastases each year in the US, there are unmet medical needs of a magnitude that pharmaceutical discovery efforts should not ignore.[1,2]

Medical need, strength of a biological hypothesis, market size, competitive landscape coupled with feasibility of discovery and development of a suitable drug are considerations that factor into whether a company pursues a drug discovery effort. For brain cancers the medical need is abundantly evident and there is no shortage of hypothesized drug targets amenable to inhibition by small-molecule drugs. Indeed, among the targets hypothesized as potential drivers of brain tumors are several kinases, a target class with demonstrated feasibility in drug discovery and development. A review of the literature reveals more than 2 dozen kinase targets that have been suggested as targets of interest for the treatment of brain cancers and for which inhibitors have advanced to clinical study (Figure 1).[3] This set of kinases consists of some for which inhibitors would be of interest in a primary brain tumor setting, some of interest where brain metastases have emerged, and others that may be of interest in both settings. Of course, some of the targets may have greater merit as legitimate drug targets than others.

Kinases have been the subject of intense interest both by pharmaceutical companies and by academic institutions for more than 30 years.[4] Through 2016, as a result of these efforts, 33 small-molecule kinase inhibitors have been approved as treatments for cancer.[5–7] Despite this apparent success, FDA approvals of kinase inhibitors for brain cancers are limited to everolimus, for the treatment of tuberous sclerosis, and alectinib, with accelerated approval, for use that includes in patients with brain metastases (anaplastic lymphoma kinase + non-small-cell

VEGFR	FGFR	HER2	MET
PDGFR	IGF-1R	b-RAF	FAK
EGFR	CDK4	MEK	Pyk2
PI3K	CDK6	PLK1	TGFR-β
mTOR	CDK1	Aurora	PIM1
ALK	CDK2	PKC	BTK
ATM	Abl	Src	AKT
ATR	Mer	AXL	WEE1

Fig. 1 Kinases for which a biological rationale exists to target for brain cancers. For a discussion of inhibitors, see Heffron.[3]

lung carcinoma).[8,9] Further, while there are approvals of numerous small-molecule kinase inhibitors whose primary targets are hypothesized as of interest for neuro-oncology (eg, epidermal growth factor receptor [EGFR], vascular endothelial growth factor receptor [VEGFR] inhibitors), there is a notable lack of regulatory approvals for such inhibitors to treat brain cancers.

The lack of regulatory drug approvals for kinase inhibitors in the brain cancer setting may not reflect that the biological rationales are flawed or invalid. It is quite possible that many of the drugs tested have not been able to sufficiently reach their target in CNS tumors, resulting in the biological hypotheses remaining untested. In order to expect efficacy from a small-molecule kinase inhibitor in any cancer setting, it must be able to engage its target within the tumor. In order for a drug to engage its target within a tumor *in the CNS*, it must first penetrate the blood–brain barrier (BBB). While there may be disruption of the BBB by some tumors, there is a growing body of literature suggesting that the barrier remains intact for some portion of the tumor or metastasis.[10–19] Glioblastoma, for instance, is a highly diffusive, infiltrative disease and in most cases much of the tumor still has an intact BBB, preventing non-brain-permeable drugs from reaching the target.[15–19] Brain metastases generally are less diffuse in comparison; however, the level of BBB disruption, and thus drug exposure in the brain tumor, is highly variable both between and within metastases.[20] As an example, within human epidermal growth factor receptor 2–positive breast cancer brain metastases, lapatinib concentration within brain metastases was found to vary from 21% to 700% of serum concentrations.[21] While individual patients might find benefit from a drug because of partial BBB disruption, many patients may not have this disruption and, for those who do, eventually tumor may progress behind intact BBB. Therefore, it is not reasonable to depend on BBB disruption to achieve consistent and sustained clinical responses, and drugs that (highly) penetrate the BBB are needed. Highlighting the fundamental requirement of free CNS penetration in the neuro-oncology setting is the emergence of CNS metastases in which the BBB provides a sanctuary from drug that is effectively treating a peripheral tumor. As examples, CNS metastases emerge as resistance to drugs known to ineffectively penetrate the BBB, including pertuzumab[22] and the small-molecule kinase inhibitor crizotinib.[23,24]

To have potential for consistent and sustained clinical response in brain tumors, drugs must penetrate the BBB. This means that some assessment of BBB penetration should be conducted preclinically to determine the merits of clinical study. In preclinical assessments of brain penetration for drug candidates, **total** (as opposed to **free**) brain concentrations or **total** brain-to-plasma concentration ratios have historically been utilized to determine the extent to which a molecule penetrates the BBB. However, in assessing a molecule's BBB penetration, simply measuring **total** drug concentrations is not sufficient to assess the level of brain penetration likely required for target engagement. The free drug hypothesis posits that it is the "free" drug (ie, the concentration of drug not nonspecifically bound to proteins or lipids) that is available to engage its target.[25,26] This means that rather than assessing total drug concentrations in the brain, research investigators should correct for nonspecific binding to brain tissue to determine if a potentially effective, or "free," drug concentration can be attained. Without this readily determined correction factor applied, an inaccurate picture of the potential of a drug candidate can result. For small-molecule drug discovery programs directed toward molecules with CNS penetration, we consider **free** brain-to-plasma ratio values >0.3 to demonstrate a significant degree of free CNS penetration. This minimal value for significance is chosen to increase confidence that the penetration measured is meaningful beyond potential experimental error. Furthermore, this target value of >0.3 exceeds the extracellular space in the brain.[27] In the author's experience with small-molecule kinase inhibitors, the total brain concentration or total brain-to-plasma concentration ratio is frequently much higher than the corresponding, and most meaningful, free drug values.

Abemaciclib serves as an illustrative example of how consideration of **total** brain-to-plasma concentration and **free** brain-to-plasma concentration ratios results in different outcomes in the assessment of brain penetration for a small-molecule kinase inhibitor (Figure 2). Researchers at

Eli Lily describe a rat pharmacokinetic study of abemaciclib in which the total brain-to-plasma concentration ratio is 1.3, suggesting complete brain penetration. However, the authors go on to note that the free brain-to-plasma concentration ratio in that study is 0.1.[28] This free brain-to-plasma concentration ratio of less than 1 is to be expected as abemaciclib is a substrate of P-glycoprotein (P-gp), a transporter highly expressed at the BBB that limits the penetration of its substrates into the CNS. When considering a free brain-to-plasma concentration ratio of 0.1 for any molecule, the plasma concentration required to achieve benefit in the CNS behind the BBB is 10-fold higher than the target concentration to achieve benefit in peripheral tumors. Clearly, a difference in target concentration of 10-fold can impact whether therapeutic hypotheses can be tested, particularly with small-molecule kinase inhibitors, which tend to have narrow safety margins. The abemaciclib example reported by Eli Lily scientists serves as an excellent illustration of what is commonly encountered in small-molecule kinase inhibitor discovery programs.

The above example demonstrates how the use of total brain-to-plasma concentration ratios can lead to misplaced optimism about the potential utility of a drug in the treatment of CNS malignancy. Without achieving adequate free drug concentration in the brain, conclusions about the merits of a therapeutic hypothesis should not be drawn. Fortunately, to assess molecules' worth for study in the CNS oncology space, free brain penetration can be measured in preclinical studies by using a combination of measured total brain concentrations (ideally at numerous time points) along with assessment of brain tissue binding. Furthermore, the likelihood that a molecule will be able to achieve high free brain penetration can be determined in vitro using cell permeability assays that assess whether or not a molecule is a substrate of P-gp or breast cancer resistance protein (Bcrp). These are 2 of the primary transporters that limit small-molecule penetration of the BBB. If a molecule is a substrate of either of those transporters, free brain penetration will most likely be limited. If a molecule is not a substrate of those transporters, then there is a potential for the molecule to achieve free brain penetration, although it may still be limited by other factors, including inadequate permeability or the action of other efflux transporters. An illustrative example is the comparison of alectinib and crizotinib. Crizotinib is a reported substrate of P-gp and in a single case was reported to achieve a ratio of CSF to free plasma of 0.03.[29,30] In contrast, alectinib is not a P-gp substrate and in a set of patients achieves near equivalent concentrations in CSF and free plasma.[31,32]

With an appreciation of the free drug hypothesis and that molecules that are substrates of P-gp or Bcrp will have limited ability to penetrate the BBB, the author assessed publicly available data for small-molecule inhibitors of kinases of interest in the brain cancer setting that have advanced to clinical trials.[3] Data for the inhibitors were reviewed to determine if **free** brain penetration was measured/calculated (as opposed to total drug brain penetration), whether or not the molecule was determined to be a substrate of P-gp or Bcrp (limiting free brain penetration) or whether a pharmacodynamic effect had been demonstrated in brain tissue (conclusively demonstrating target engagement and, therefore, free brain penetration).

For the significant majority of small-molecule kinase inhibitors of targets of interest for CNS cancers that have advanced to clinical trials, including those studied in trials enrolling patients with brain cancers, there is insufficient evidence to expect high free drug penetration of the BBB (Table 1).[3] Most of the kinase inhibitors reviewed that have advanced to clinical trials are substrates of P-gp, hence expected to have limited brain penetration. For many, in

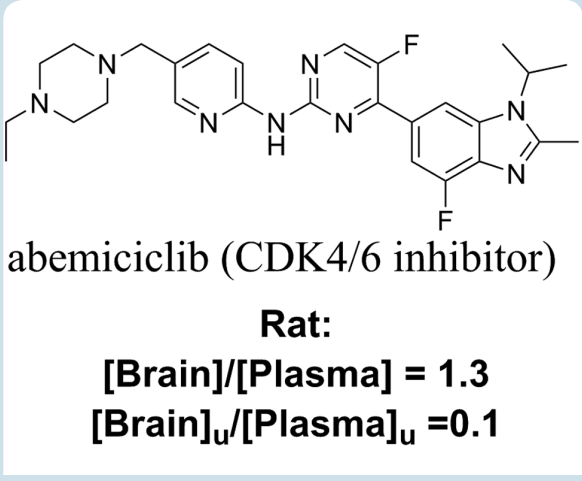

Fig. 2 Comparison of total and free brain-to-plasma concentration ratios in rat for abemaciclib.[28]

Table 1 Summary of kinase targets of interest for neuro-oncology grouped according to whether known CNS penetrant clinical inhibitors are available (see Heffron[3] for details)

Kinase Targets with CNS Penetrant Clinical Inhibitors Available	Kinase Targets without Known CNS Penetrant Clinical Inhibitors
EGFR	VEGFR
PI3K/mTOR	AKT
ALK	IGF-1R
HER2	CDK1/2
MEK	b-RAF
Abl/Src	PLK1
BTK	Aurora
	PKC
	c-MET
	FAK/Pyk2
	TGFR-b
	PIM1
	ATM
	Mer
	AXL
	FGFR
	CDK4/6

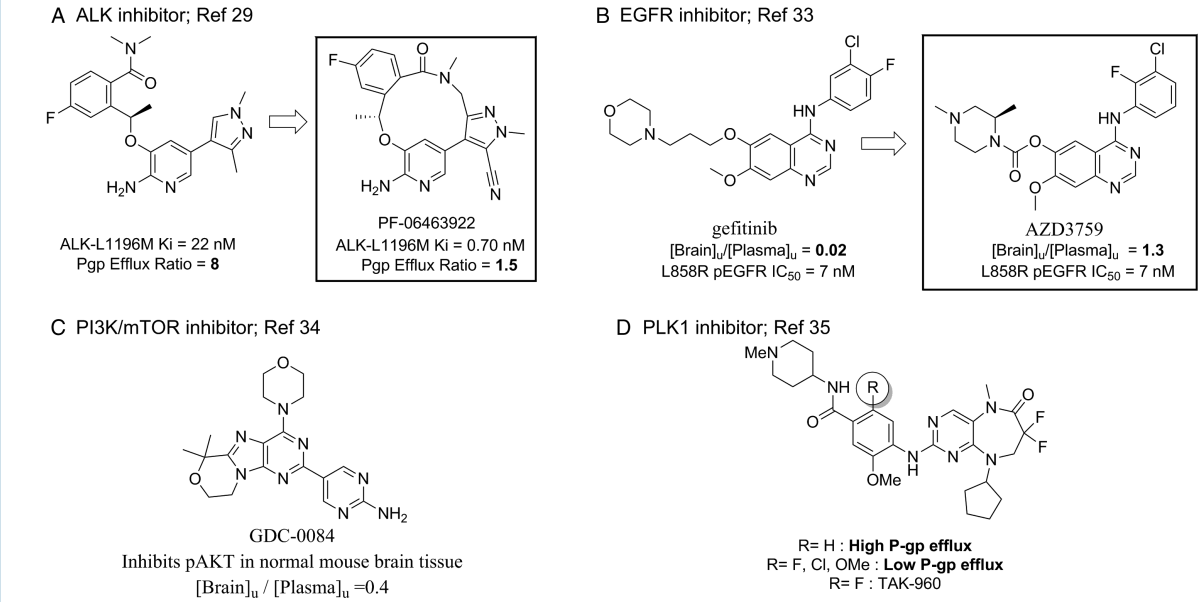

Fig. 3 Examples of kinase inhibitors for neuro-oncology designed and demonstrated to achieve high free brain penetration in preclinical studies (A–C) or designed and demonstrated to not be a substrate of P-gp (D). $[Brain]_u/[Plasma]_u$ refers to the ratio of unbound, or free, brain and plasma concentrations in rodent pharmacokinetic studies.

vivo preclinical studies have clearly demonstrated that brain penetration is limited by the action of P-gp. For another group of the kinase inhibitors reviewed, inadequate data exist to determine whether or not it is reasonable to expect meaningful free brain penetration. Of course, the absence of data does not allow for a conclusion that a molecule is a P-gp substrate and has limited potential for CNS penetration. However, in this author's experience with kinase inhibitors, those that are not P-gp substrates are the exception rather than the rule, and the burden of proof should fall on the need to demonstrate free brain penetration rather than to expect it.

There are many reasons why small molecules might fail in clinical studies. An inadequate pharmacokinetic profile may limit exposure. Toxicity might prevent achievement of drug exposure needed for efficacy. For the treatment of brain cancer, however, whether or not sufficient free drug concentration in the brain to provide efficacy can be realized is an additional consideration. So, while many kinase inhibitors have failed in clinical trials for the treatment of brain cancers, if those molecules were not capable of free penetration of the BBB, the biological hypotheses likely were not tested. That is, even if a drug can provide benefit in the treatment of peripheral tumors, it may not have potential to be an effective treatment for brain cancers simply because it might be restricted from achieving adequate concentrations in the CNS. In this scenario, it does not mean that the target of that inhibitor is not a valid therapeutic target in the treatment of CNS malignancy. Rather, to fully evaluate the therapeutic hypothesis, freely BBB-penetrating inhibitors are needed that also have the pharmacokinetic and safety profiles to enable meaningful study. Furthermore, as most cancers require combination treatment, in the neuro-oncology setting there may be the need for multiple brain penetrant partners in order to derive a benefit.

Encouragingly, in recent years there have been several small-molecule kinase inhibitors for brain cancers identified as capable of achieving meaningful free brain penetration.[29,33,34] For these molecules, free brain penetration was assessed either through measurement of free drug levels in the brain or by demonstrating a pharmacodynamic effect in the CNS (Figure 3A–C). Another recent report describes kinase inhibitors designed specifically to avoid transporter efflux at the BBB, with the goal to achieve high free drug penetration in the brain (Figure 3D).[35] Each of these examples highlights that the drug discovery field is beginning to recognize the importance of achieving **free** brain penetration to have an increased chance at effectively treating CNS cancers. Additionally, these examples demonstrate that physicochemical property optimization of kinase inhibitors during the discovery phase can lead to drug candidates that are potent and selective and have desirable pharmacokinetic properties that include **free** brain penetration. That is, the types of molecules required to enable clinical evaluation of hypotheses for the treatment of brain cancer can be realized.

By acknowledging the need for penetration of free drug across the BBB to treat CNS malignancy, drug discovery programs have the potential to design effective drug candidates that can evaluate therapeutic hypotheses. New, freely BBB-penetrating kinase inhibitors are

needed to adequately assess the value of inhibiting particular kinases for the treatment of CNS cancers. Prior to advancement of new molecules to study for the treatment of brain cancers, candidates should be assessed for their ability to **freely** penetrate the BBB. Doing so should result in more effective clinical investigations and greater benefit to patients.

Conflict of interest statement. Timothy Heffron is an employee of Genentech, Inc and a shareholder of Roche.

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