Published in final edited form as:

Clin Cancer Res. 2016 December 15; 22(24): 5953–5955. doi:10.1158/1078-0432.CCR-16-2035.

Therapeutics for Brain Metastases, Version 3

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Summary

The role of blood-brain barrier (BBB) permeability in the efficacy of brain metastasis therapeutics is debated. A BBB-permeable and a BBB-impermeable compound were compared in a melanoma brain metastasis model using imaging through a cranial window. Only the BBB-permeable compound inhibited both the ~30% permeable metastases and the ~70% impermeable metastases.

In this issue of *Clinical Cancer Research*, Osswald *et al.*(1), in the lab of Dr. Frank Winkler, use cutting edge technologies to visualize melanoma brain metastases, their permeability, and the efficacy of brain-permeable versus brain-impermeable phosphoinositide-3 kinase (PI3K) inhibitors. Brain metastases are devastating complications of melanoma, lung cancer and breast cancer, causing both physical and neurocognitive declines. Treatments are palliative, including radiation therapy, surgery and steroids. The role of drugs in brain metastasis therapy is murky, due to the debated role of the blood-brain barrier (BBB), the protective lining of brain blood vessels, and the poorly understood blood-tumor barrier (BTB), whatever remains of the BBB once a metastasis forms. Increasingly, good, hematogenous model systems are providing insights into the drug permeability/efficacy quandary, and can guide both drug development and clinical testing.

Using an *in vivo* model of experimental brain metastasis of the A2058 melanoma cell line, Osswald *et al.* incorporated two photon imaging through a cranial window and pioneering quantitation methods. A2058 cells were injected into the left ventricle of the heart and colonized the brain among other organs. Sodium fluorescein dye was injected after brain metastasis formation, and the ratio of dye in vessels versus metastases used to indicate permeability. Non-permeable metastases were 71.2% of the total, and exhibited a slower growth rate (Figure 1A). Permeability did not change with time.

Animals harboring brain metastases, permeable and impermeable to dye, were then randomized to vehicle or two PI3K inhibitors: GNE-317, a compound not in clinical testing, but with brain permeability; and GDC-0980, a clinical compound without significant brain permeability. Initial *in vitro* experiments demonstrated that the two compounds had comparable effects on signaling, and pharmacokinetic experiments identified doses and schedules to equalize their AUCs *in vivo*. Using the cranial windows, a total of 45 metastases were serially imaged for growth rate, morphological signs of apoptosis,

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Conflicts. Dr. Steeg receives research funds from Genentech.

dormancy, and blood vessel proximity. The daily growth rate of permeable metastases was reduced by both compounds, but only the BBB permeable GNE-317 reduced the growth rate of the majority impermeable metastases (Figure 1B). The trends were confirmed by measuring tumor cell apoptosis: morphological nuclear apoptosis was apparent in the permeable lesions in response to either drug, and in the non-permeable lesions only in response to GNE-317. Interestingly, most of the apoptosis was distant from blood vessels, indicating that, once through the BBB/BBB, drug effect extended away from the vessels. This observation also suggests that the perivascular niche may confer survival signals. In one animal, dormant tumor cells were visualized and tracked. These cells remained stable and were non-permeable through 30 days post-injection; upon treatment with GNE-317, the dormant cells died.

Multiple important issues will contribute to the development of better therapies for brain metastases, including the identification of molecular drivers and drug resistance pathways, and dealing with brain permeability issues. With regard to brain permeability and drug efficacy, the literature has been thorny. We break it down into three versions:

v1. Confusion

Conflicting observations yielded no generalizable insights. Brain metastases must be permeable because they image with gadolinium. Brain metastases turn blue when the mouse is injected with trypan blue, so they are permeable (2). Therefore, brain permeability is not an issue. Then why does chemotherapy, even chemotherapy with activity in the metastatic setting, not work in brain?

v2. Metastases are heterogeneous, mostly impermeable

Using different methodologies, multiple independent groups have observed heterogeneous permeability of hematogenous brain metastases. The laboratories of Drs. Quentin Smith and Paul Lockman used multiple mouse models bearing brain metastases of breast cancer, injecting mice with dyes and radiolabeled drugs, and then perfusing the mice to clear the vasculature. Using quantitative imaging, uptake of dyes and drugs was above that of the BBB in ~85% of metastases. The first answer is that most brain metastases were permeable, but the next question may be more important: are they permeable enough? Only metastases with the highest uptake levels, ~50-fold above the BBB representing ~10% of lesions, demonstrated an apoptotic response to paclitaxel (3). The labs of Drs. Paula Foster and Ann Chambers used gadolinium contrast-enhanced MRI and high-resolution anatomical MRI to image permeability in a model system of HER2+ brain metastasis of breast cancer. Heterogeneous permeability was also observed (4). In separate work from the same group, two of three breast cancer brain metastasis model systems even showed heterogeneous permeability to gadolinium, suggesting that we only detect some of the brain metastases that exist (5). In other models, permeability was heterogeneous based on metastasis size (6). Osswald *et al.* confirm these observations in a melanoma model system, with 71% of lesions nonpermeable. The mouse model findings of heterogeneity were confirmed in a human presurgical study of capecitabine and lapatinib uptake (7). The data support an emerging

consensus that the permeability of brain metastases is heterogeneous, with a significant percentage being poorly permeable.

v3. Brain permeable drugs work better

The study of Osswald *et al.* provide a significant advance in the field, testing side by side brain permeable and -impermeable compounds targeting the same molecule (PI3K). Only the brain permeable compound was able to inhibit the growth of impermeable lesions, the majority of lesions in the brain. Interestingly, when metastases were dichotomized into micrometastases and macrometastases (50 tumor cells as the cut-off), the brain permeable compound shrank the micrometastases but only slowed the growth of the macrometastases. These data bolster previous arguments that prevention of brain metastases by drugs may be more efficacious than treatment of larger established lesions (8, 9). The brain permeable compound was also active against rare impermeable dormant tumor cells.

There are potentially many definitions of the term “brain permeable”. The work of Smith and Lockman, and now Osswald *et al.*, support a unifying explanation: “Brain permeable” in translational research means sufficient uptake for a drug response.

What is the future?

Future studies will apply these careful measurements to other brain metastasis model systems. More translational model systems are needed to reflect the heterogeneity of brain metastases. It is unknown at this point if implantation models provide valuable BBB permeability data, after intracranial injection of a bolus of tumor cells or implantation of a chunk of tissue. Future studies will also need to clarify the target: Do drugs need to be brain (BBB) permeable, or BTB permeable? Are they different? In theory, permeation of the BTB, with less permeability toward the BBB, could minimize toxicities to the normal brain.

There are few truly brain permeable drugs in the clinic. As measured by brain uptake in mice, or CSF concentrations in humans, targeted drugs such as gefitinib, erlotinib, lapatinib, vemurafenib and crizotinib are partially permeable at best (10-12). To date, while responses and progression free survival advantages have been observed, disease progression is common and it is hoped that improved permeability will increase drug efficacy. Clearly, more brain permeable, effective drugs are needed. Incorporation of brain passive permeability criteria (lipid solubility, hydrogen bonding, molecular mass) into the prioritization in drug development would be a straightforward first step. Both brain metastasis treatment and prevention clinical strategies need consideration.

Acknowledgments

Support. This work is supported by the Intramural Program of the National Cancer Institute and grant W81XWH-06-2-0033 from the DOD BCRP.

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Figure 1. The role of brain permeability in the efficacy of therapeutics for brain metastases
A2058 melanoma cells were injected into the left cardiac ventricle of mice and brain metastases formed. **A.** Sodium fluorescein dye (green) was injected before necropsy and its uptake into lesions used as a measure of brain metastasis permeability. Only ~29% of the melanoma metastases were permeable to dye (green clouds). **B.** Mice harboring both permeable (green clouds) and impermeable (none) brain metastases were randomized to vehicle or two PI3K inhibitors, GDC-0980 which is brain impermeable, or GNE-317 which has demonstrated brain permeability. Both drugs inhibited the growth of permeable metastases. Only the brain permeable GNE-317 inhibited the growth, and caused tumor apoptosis (gray tumor cells, X), of impermeable metastases. The data confirm a functional contribution of brain permeability to drug efficacy for brain metastases.