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Brain access of monoclonal antibodies as imaged and quantified by ^{89}Zr-antibody-PET: perspectives for treatment of brain diseases

Invited Perspective on JNM manuscript titled, "PET imaging of intra-arterial [^{89}Zr]bevacizumab in mice with and without osmotic opening of the blood-brain barrier: distinct advantage of intra-arterial delivery" by W.G. Lesniak, C. Chu, A. Jablonska, Y. Du, M. Pomper, P. Walczak, and M. Janowski

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In the search for effective therapies against central nervous system (CNS) diseases, it is questioned whether sufficient intra-lesional drug concentrations can be reached upon systemic administration. This is especially true for biologicals such as monoclonal antibodies (mAbs), which are relatively large in size. Few patient data is available on the passage of mAbs over an intact blood-brain barrier (BBB) because reliable, time-effective and non-invasive methods to quantify drug concentration in the brain are lacking. In this issue of The Journal of Nuclear Medicine, Lesniak et al. make use of PET imaging of the mAb bevacizumab labeled with the long-lived positron emitter Zirconium-89 (^{89}Zr, $T_{1/2}$ = 78 hrs) to quantify uptake in different brain regions in mice for extended time periods of days (*1*). With this study they provide avenues to overcome two major obstacles in the development of effective treatment strategies for CNS diseases: (i) adequate drug concentrations by chemical opening of the BBB, and (ii) efficacious drug monitoring by in vivo imaging and quantification of brain uptake over prolonged time.

The prospects for patients with glioblastoma, for example, have not improved for decades with less than 5% of patients surviving 5 years following diagnosis (*2*). This is in contrast with spectacularly increased survival of patients with specific hematologic and solid malignancies outside the brain. Historically, lack of success from chemotherapeutic strategies in brain tumors was presumed to be due to chemoresistance of cancer cells, resulting in studies investigating high-dose or multidrug regimes, as well as targeted therapies such as mAbs. After lack of response in patients, the reigning paradigm of intrinsic tumor chemoresistance shifted to a supposed delivery problem over a blood brain- and brain tumor barrier. Non-invasive methods to quantify drug concentration in brain and tumor, however, are lacking and theories regarding BBB integrity are mainly based on magnetic resonance imaging (MRI) studies using contrast agents. Contrast enhancement is assumed to reflect extravasation through a locally disrupted BBB. BBB disintegration is seen in many CNS disorders such as encephalopathy, multiple sclerosis, Alzheimer disease, Parkinson's disease, seizures, stroke and trauma (*3*). In brain

tumors, especially in lower-grade diffuse gliomas with extensive infiltration in the normal brain, the BBB is probably largely intact, which is reflected by limited contrast enhancement upon gadolinium administration. Regions of contrast enhancement, on the other hand, are associated with formation of disordered and highly permeable tumor neovasculature indicating higher malignancy and consequently shorter survival. Since contrast enhancement is only an indirect proof of possible drug penetration through the BBB, direct methods to quantify drug concentrations in brain are urgently needed (*4*).

In normal, non-diseased brain, the BBB meticulously regulates inter- and paracellular transport of substances via passive diffusion and active transport mechanisms. Transport over the BBB depends on both the physicochemical properties of the drug itself (e.g., lipophilicity, molecular weight, and charge), as well as its affinity for in- and efflux transporters and receptors (e.g., ATP-binding cassette transporters and ABC-transporters). El-Khouly et al. recently developed a theoretical model including all these parameters for a long list of commonly-used anti-cancer drugs to review their likeliness of passage over an intact BBB (*5*). They predicted that only few drugs, 8 of 51 (15%), actually penetrate the BBB upon systemic administration, which may explain the lack of efficacy in clinical trials thus far. Yet again, this model is only a theoretical model, and direct methods to quantify actual concentration in brain over time are urgently needed.

To overcome the presumed drug delivery obstacle in the brain, many treatment strategies for CNS diseases are directed at disrupting, passing or bypassing the effective BBB. Disruption has been tried chemically, by drugs that influence passive diffusion (e.g., bradykinin or cereport, mannitol, regadenoson, and borneol) or active transport mechanisms (e.g. elacridar), or externally such as by radiotherapy, ultrasound or microwaves. Passing the BBB has been tried via viral vectors, nanoparticles, liposomes, exosomes, and by transporter or receptor ligands. Novel technical approaches such as convection-enhanced delivery are being developed to bypass the BBB. In these studies, small molecular agents were often used, and biological agents of larger molecular weight, like mAbs, only very limited.

As for mAb therapy, evidence for a drug delivery problem over an intact BBB is provided by the fact that mAbs are used with increasing success against various solid tumors outside the brain, such as lymphoma, breast - and colorectal cancer, but disappoint for use in brain tumors and other brain diseases. Even more so, in breast cancer patients, tumor reponse was observed after systemic mAb treatment, while their intracranial metastases did not respond. Nonetheless, mAbs are broadly explored, not only for cancer but also for other CNS diseases such as Alzheimer's, Parkinson's, multiple sclerosis, and stroke because of their specificity and high-affinity for critical disease targets. Moreover, mAbs can be labeled with radioactive

isotopes to allow the study of functional behavior in vivo. By labeling mAbs with for instance the long-lived radionuclide ^{89}Zr, whole body biodistribution as well as selective brain uptake can be visualized and quantified by PET at high sensitivity and resolution over days, rendering this approach superior over other molecular imaging approaches. Nevertheless, a limiting factor of clinical ^{89}Zr-antibody-PET (also referred to as 89Zr-immuno-PET) is the radiation burden to patients, which is relevantly reduced by the introduction of high-sensitivity whole body PET scanners. A recent review summarizes the distinguished advantages as well as the first 15 clinical trials using ^{89}Zr-antibody-PET (*6*). One of these studies was the first to apply ^{89}Zr-labeled bevacizumab PET imaging in pediatric patients with diffuse intrinsic pontine glioma (*7*), demonstrating substantial variability in the level of ^{89}Zr-bevacizumab tumor uptake within the contrast-enhancing MRI areas. This indicated the added value of ^{89}Zr-antibody-PET in explaining the poor prognosis of these patients by BBB integrity. Additionally, ^{89}Zr-antibody-PET showed value in women with HER2-positive metastatic breast cancer (*8*). Here, an 18-fold higher uptake of trastuzumab was observed in brain metastasis as small as 0.7 mm and previously undetected by MRI, compared to normal brain tissue. This is in line with growing evidence that brain metastasis could disrupt the BBB once the diameter exceeds 0.5 mm (*9*). The evidence that mAb delivery to brain metastasis is possible supports the use of trastuzumab therapy. This is an important finding because there is an increase in brain metastasis among women with HER2-positive breast cancer as a consequence of improved systemic therapy. Visualization and quantification of mAb uptake by PET could decrease the risk of experiencing morbidity and mortality as a result of uncontrolled brain metastases, which often occurs at a time when the primary tumor is apparently under control.

Lesniak et al perform ^{89}Zr-bevacizumab PET imaging in non-tumor bearing mice using four drug delivery strategies: (i) intra-venous infusion with intact BBB or (ii) with BBB-opening by administering mannitol 15 minutes prior, versus (iii) intra-arterial (IA) infusion with intact BBB or (iv) BBB-opening by co-administion of mannitol. Of note, gradual linear increase in ^{89}Zr-bevacizumab concentrations was observed until at least 24 h post infusion, contrary to immediate clearance from brain of many nanoparticles or small molecules. Most importantly, the fastest and highest brain uptake was measured from IA administration of bevacizumab in combination with BBB-opening. Finally, negligible drug concentrations were measured in the contralateral hemisphere indicating selective delivery and toxicity.

The results of this study are intriguing, however, a prominent question arises: Why did the authors choose to renew this therapeutic strategy? IA treatment of brain diseases has been attempted since the 1950s and the first phase I studies on osmotic opening of the BBB by mannitol date back to 1979 (*10,11*). These strategies have since not been adopted for patients after lack of success in clinical studies over the next decades. On the one hand, the endovascular

treatment approach remains appealing, because it is minimally-invasive and has proven to be safe with a complication rate of 0.30% (*12*). On the other hand, after IA delivery of chemotherapy, vascular and neurologic toxicity was reported with visual loss, stroke, and leukoencephalopathy compared to the same drug dose given systemically, although these toxicities were attributed to the specific drugs that were administered (*13*).

What do we learn from the study performed by Lesniak et al? From decades of previous investigations we learned that IA delivery of (neuro)toxic drugs for brain diseases is a promising yet risk full treatment approach. Selecting the right drug and right dose are crucial, especially without proven clinical efficacy of IA drug delivery. Currently there are four active open phase I/II clinical studies combining osmotic BBB disruption using mannitol with IA infusion, three using mAbs at therapeutic dose: NCT00303849; NCT02861898; NCT02800486; NCT01269853. None of the trials, however, include actual drug concentration measurements. We learned from Lesniak et al. that similar studies should include actual drug concentration measurements that is feasible with minimally-invasive ^{89}Zr-antibody PET. Ideally, all phase I/II clinical trials should be primarily directed at obtaining information on drug distribution to indicate potential toxicity and on actual disease targeting to indicate potential efficacy. Today, newly-developed Phase 0 pharmacokinetic (PK)/pharmacodynamic (PD) studies for brain diseases largely depend on invasive procedures at single time points, such as lumbar puncture or brain biopsy. Lesniak et al. show the added value of ^{89}Zr-antibody-PET imaging over time as a potential imaging biomarker for drug toxicity and efficacy. This can facilitate better understanding of earlier lack of success and can prevent potentially toxic, expensive and useless exposure of patients in clinical trials. Moreover, molecular drug imaging studies could enable precision medicine by selecting the right drug at the right dose for the right patient and guide drug development to delivery behind the BBB with great promise for future therapy of CNS diseases.

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