Monoclonal antibodies (mAbs) are used with increasing success against many tumors, but for brain tumors the blood-brain barrier (BBB) is a special concern. The BBB prevents antibody entry to the normal brain; however, its role in brain tumor therapy is more complex. The BBB is closest to normal at micro-tumor sites; its properties and importance change as the tumor grows. In this review, evolving insight into the role of the BBB is balanced against other factors that affect efficacy or interpretation when mAbs are used against brain tumor targets. As specific examples, glioblastoma multiforme (GBM), primary central nervous system lymphoma (PCNSL) and blood-borne metastases from breast cancer are discussed in the context of treatment, respectively, with the mAbs bevacizumab, rituximab and trastuzumab, each of which is already widely used against tumors outside the brain. It is suggested that success against brain tumors will require getting past the BBB in two senses: physically, to better attack brain tumor targets, and conceptually, to give equal attention to problems that are shared with other tumor sites.

Over time, we have become more sophisticated about both the bullet and its magic. For tumors outside the brain, long-term remissions can indeed be achieved, but not all eligible patients respond, responses may be short-lived and side effects can be limiting. Many of the evolving insights and adaptations apply very generally to many different therapies or to tumors at many sites. Additional insights apply and new insights are still needed for tumor in the brain.

In applying mAb therapy to brain tumors, both expectations and interpretation are colored by awareness of the blood-brain barrier (BBB). A striking example is seen when tumors in the bodies of breast cancer patients respond to systemic mAb treatment, but then metastases appear in the brain. The reason most often suggested for this phenomenon is that the BBB must be blocking access to the brain metastases, making the brain a sanctuary site; however, the full explanation must be more complex. Accumulating experience suggests that systemic antibody can be beneficial for patients with brain metastases or with other targets in the brain. How can this be the case if access is blocked by the BBB? If access is not in fact limiting, how is late failure in the brain to be explained, especially when it occurs in parallel with tumor control at other sites?

The questions above set the context for this review. To address them, brain tumors (the target), antibodies (the magic), how antibodies attack tumor (the bullet) and how they reach it (the barrier) are reviewed in turn. With this as background, practical experience with mAbs for brain tumor targets is re-visited (the findings) and ways to go forward are suggested (the future).

As will be brought out, the special problems posed by the BBB are balanced by more general concerns. Indeed, one aspect of “getting past the BBB” will be to give greater attention to problems that are shared with other tumor sites.

### Introduction

An early hope for monoclonal antibodies (mAbs) was that they would serve as tumor-specific magic bullets in two ways. As bullets, they would move through the blood to reach and attack tumor targets. The exquisite specificity of a single antibody would provide the magic. Experience with tumors outside the brain has begun to justify this hope. Among the best-studied examples, the mAbs trastuzumab (Herceptin®), rituximab (Rituxan®) and bevacizumab (Avastin®), are now approved as part of the standard therapy for appropriate forms of breast cancer, glioma, primary central nervous system lymphoma, brain metastases, respectively, as well as other types of cancer.

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**Key words:** monoclonal antibody, brain tumor, immunotherapy, glioma, primary central nervous system lymphoma, brain metastases, bevacizumab, rituximab, trastuzumab

**Abbreviations:** ADCC, antibody-dependent cell-mediated cytotoxicity; BBB, blood-brain barrier; CDC, complement-dependent cytotoxicity; CNS, central nervous system; CSF, cerebrospinal fluid; ECM, extracellular matrix; GBM, glioblastoma multiforme; MS, multiple sclerosis; PCNSL, primary central nervous system lymphoma; PVS, perivascular space; VEGF, vascular endothelial growth factor
As such, GBM has been the focus of much work with mAbs, as (GBM), is the most common and aggressive type in adults. The tumor mass and the infiltrative component present differ-
vantly in pre-clinical work.

Microscopic tumor (or micro-tumor), tumor too small to be readily imaged by conventional methods, is an important component of many brain tumors, including those stressed here. For GBM or other glial brain tumors, infiltrative tumor is known to remain after a main tumor mass has been removed,12,15 PCNSL normally appears as a diffuse B-cell lymphoma17 and blood-borne tumor from other organs first enters the brain as micro-metastases.

For the many cases where it is known that micro-tumor is likely to be present somewhere in the brain, but not exactly where, localized therapies are not appropriate. This increases interest in agents such as mAbs that are less inherently toxic than the conventional therapies,24,25 and so safer for widespread delivery. For tumor outside the brain, a complementary attraction is that most therapies are thought most likely to succeed against micro-tumor, as opposed to larger masses.26 A confounding factor in the brain is that the BBB is closest to normal, and therefore most effective at blocking antibody access, at micro-
tumor sites. A related problem is that, although the importance of brain micro-tumor is widely acknowledged, it is rarely targeted explicitly in pre-clinical work.27,28

The challenges of treating brain tumor targets are well illustrated by experience with the mAbs listed in Table 1. Before turning to the clinical findings, it is useful to briefly review prop-
erties of the mAbs themselves.

Table 1. Tumor/antibody combinations emphasized in the text

<table>
<thead>
<tr>
<th>Brain tumors discussed</th>
<th>Antibodies discussed</th>
<th>Target antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>General type</td>
<td>Specific example</td>
<td>Name</td>
</tr>
<tr>
<td>glioma</td>
<td>GBM</td>
<td>bevacizumab (Avastin)</td>
</tr>
<tr>
<td>lymphoma</td>
<td>PCNSL</td>
<td>rituximab (Rituxan)</td>
</tr>
<tr>
<td>metastatic</td>
<td>breast cancer</td>
<td>trastuzumab (Herceptin)</td>
</tr>
</tbody>
</table>

*GBM, glioblastoma multiforme; **PCNSL, primary central nervous system lymphoma; **VEGF, vascular endothelial growth factor; **CD20, common B-cell antigen; **Her2, human epidermal growth factor receptor 2

Figure 1. Two patterns of tumor growth in the brain. Tumor often grows around blood vessels (left), but some tumors can also infiltrate the brain parenchyma (right).
The Magic: What Tumor-Specific Can Mean

Although antibody specificity is indeed exquisite,29 few determinants are limited to tumor cells (Fig. 2).24,30 Fortunately, shared molecules can serve as practical tumor targets and this is true for each molecule targeted by the mAbs in Table 1.

Each B-cell lymphoma expresses a unique idiotype, and this may well be the target of choice in the long term.31 Rituximab, in contrast, recognizes a common B-cell antigen, CD20, that is expressed by both normal and neoplastic B cells. At present, the advantage of rituximab is that it can be used for many different patients, coupled to the fact that the depletion of normal B cells can be tolerated because existing antibody and antibody-forming plasma cells are spared, other protective mechanisms remain active and B cells are eventually replaced.32,33

Although more restricted antigens are known for GBM,34 there has been enormous interest in using bevacizumab to target vascular endothelial growth factor (VEGF), which promotes angiogenesis for tumors, but also in normal wound healing.35 Trastuzumab targets Her2, which is overexpressed in many breast cancers, but also expressed by normal cells.1,24,36 In practice, each of these three mAbs has been used successfully against tumor outside the brain1-8 and, increasingly, is being used against brain tumor targets.

Although targets need not be limited to strict tumor-specific antigens, cross-reactions may cause problems for individual patients. More generally, the problem of resistance is increasingly acknowledged.2,4,12,37,38 For any given mAb, a tumor cell can escape attack at many levels, from its expression of the target determinant, to its susceptibility to the final effector mechanism.12,31,37-39 Probing the basis of resistance is complicated by uncertainty as to which, among many possibilities, are the most important effector mechanisms for a given mAb, tumor and site.

The Bullet: What Antibodies Can Do

Antibodies can lead to death or arrest of a tumor target in a great and growing variety of ways, with new antibody-mediated functions still being identified. Antibodies can directly block activity of a target molecule simply by binding to it, through the antibody variable region,36,40 while additional functions are brought to bear if the constant (Fc) region is engaged. The benefits of Fc binding to elements of the complement cascade or cell-bound Fc receptors are well known.36,40 More recently appreciated is that another kind of Fc receptor, FcRn, binds to an antibody in a way that protects it from degradation. This contributes to the prolonged serum half-life of an antibody, as compared to that of most other proteins.36,41

An antibody need not attack tumor cells themselves. Bevacizumab, which is intended to block development of a tumor’s blood supply, is an example.35 Also, the antibody does not necessarily need to attack its target in the brain. Bloodborne tumor or other targets may be attacked before they reach the brain. Complexes between an antibody and its target antigen can stimulate the patient’s own immune response or mAbs can modulate an ongoing response.30,38,42 Complementing these spontaneous activities, additional effector mechanisms come into play when mAbs are coupled to agents, such as radionuclides or toxins,24,40 or larger molecules or particles (liposomes, nanoparticles),24,45-47 so that the mAb serves a targeting function and the other component provides (or adds to) the attack mechanism. The sequence of the antibody protein itself may be modified to alter target binding or constant region functions,42 or avoid having the antibody recognized as a foreign protein by the patient’s immune system. All three antibodies discussed here were modified to reduce such recognition (Table 1).40 The variety of possible effector mechanisms is multiplied as antibodies are used in combination with other agents or modalities.37 Of particular relevance for brain tumor, radiotherapy is thought to alter the BBB in ways that increase antibody access to tumor sites.23,44

Even where antibody-mediated therapy has been most successful, the key effector mechanisms are not yet known.2,6,32,35,36,38,40 Not only is there a wealth of possibilities, but the balance of effector functions may differ at different tumor sites.12 The frequent use of combination therapies and the potential long-term effects of previous treatments further increase the difficulty of defining the effect of a given antibody. There may also be uncertainty as to where an antibody exerts its effects, for example, whether metastatic tumor is attacked at its source, en route or at its final site. In the brain, interpretation is further impeded by the difficulty of directly analyzing the tumor site. These uncertainties, in turn, complicate interpretation of the role of the BBB.
The Barrier: How Entry to the Brain is Controlled

The ability of the BBB to block passive entry of therapeutics into the normal brain is well known; however, understanding of the BBB, even in the normal brain, is still evolving. The BBB is dynamic and can be manipulated; unfortunately, it is not the only impediment.

Entry to normal brain. What impedes entry. In the brain parenchyma, the anatomical BBB is formed by specialized tight junctions between the endothelial cells of cerebral micro-vessels, together with astrocyte endfeet that abut the endothelium, adjacent pericytes and a characteristic composition of the extracellular matrix (ECM). This anatomical barrier is complemented by a physiological barrier that includes reduced pinocytosis by the endothelial cells (as compared to other tissues), degradative enzymes and transporters that act as efflux pumps to actively return many kinds of molecules, including many drugs, to the blood. Other CNS compartments have variations of these properties, so that the brain environment remains controlled.45–47

What allows entry. As effective as the BBB is in preventing unregulated entry of substances from the blood, it is equally effective at selectively permitting entry of necessary components. Active transporters import nutrients and regulatory molecules.44-47 One approach to delivering agents to the brain is to exploit these transporters.44,45

Of special relevance for antibody therapeutics, FcRn, the Fc receptor that protects antibodies from degradation in serum, is highly expressed on brain vessels.41 In other organs, another function of FcRn is to transport antibody across tissue barriers. For the brain, a role in removal of harmful antibody from the brain has been suggested,41,42 but the actual roles are not yet known. In other tissues, FcRn-mediated transport is bi-directional, and the predominant direction can be modified experimentally.48 It is intriguing to consider whether FcRn might also act to bring antibody into brain tumor sites—or might be exploited for that purpose.

Entry to tumor sites. Not static. The BBB is dynamic, its properties specified and maintained by reciprocal interactions with adjacent cells. BBB properties change during development and other normal processes, as well as in the context of inflammation or other pathology,45-47,49 including tumor growth.44,50 The BBB may be essentially normal at micro-tumor sites; as the tumor grows, the BBB is progressively disrupted. Ultimately, new vessels may be formed and the tumor itself modulates their properties.50 Indeed, for brain metastases, tumor-adjacent vessels may resemble abnormal versions of vessels in the tissue of origin, rather than brain barrier vessels.44,50

Not the only impediment. When primary or metastatic brain tumor can be imaged by a contrasting-enhancing agent, typically gadolinium, the vessels must be at least leaky enough to permit the agent’s extravasation. Unfortunately, this does not necessarily imply effective delivery of mAbs. Even in the normal brain, the distribution of a given molecule will vary with its size, shape, charge, composition (such as lipophilicity) and the extent to which it binds to blood components or tissue.36,45,46 As two examples of the relevance of these factors, antibodies are much larger than gadolinium50 and many drugs bind tightly to serum proteins.50 An important consequence is that the distribution of a contrast-enhancing agent does not necessarily reflect the distribution of therapeutic agents.

Tumor-associated vessels may be leaky, but are also tortuous and chaotic, so that, even if blood-borne molecules do leave the vessel, they may not be evenly distributed within the tumor. Factors such as an unfavorable pressure gradient and the nature of the extracellular matrix can impede the ability of therapeutics, including antibodies, to become well-distributed within a tumor mass, while the center of a large tumor can act as a sink.26,44,50,51

In GBM in particular, blood-borne agents will accumulate in the necrotic center, with its disrupted BBB, rather than at the infiltrative edge, where the BBB is more like that of normal brain.50 Properties of the tumor cell itself can further impede antibody binding or efficacy. Most of these factors are not unique to brain tumors; rather, they can impede the distribution or activity of antibody or other therapy against solid tumor at any site.66-68

In the brain, interpretation of antibody levels is hampered by the difficulty of taking local measurements. Instead, the concentration of therapeutic agents is often followed by measuring levels in cerebrospinal fluid (CSF). This approach does not take into account anatomic and metabolic heterogeneity even within the normal brain, and potential inaccuracy is additionally compounded by heterogeneity among tumor sites.44,46,49,52 Even at a single site, antibody access can change with time. As tumor either responds to therapy or continues to grow, the BBB itself, as well as the other impediments to antibody access, will change accordingly.17,18,53

The Findings: Experience with Three Brain Tumor/Antibody Pairs

The topics discussed so far provide a context for reviewing clinical experience with mAbs in brain tumor therapy. Three of the best-studied antibody/target combinations (Table 1) illustrate specific details.

Bevacizumab and GBM. The original intention was that bevacizumab would bind VEGF and so attack the highly vascular main tumor mass indirectly, by depriving it of its blood supply.15,35,34,54 In practice, the antibody does affect tumor blood vessels, reducing their leakiness and other abnormal properties. Benefits of this vessel normalization include reduced edema and intracranial pressure, which permits reduced steroid use,15,54 and perhaps also protection against radiation necrosis.55,56

The effect on tumor growth per se has been hard to interpret. Because it affects blood vessels, bevacizumab also affects extravasation of the contrast agents used to image a tumor mass. In this situation, improvement in the radiographic image can be misleading: It may simply reflect reduced extravasation of the contrast agent rather than tumor control.15

The disseminated components of GBM present complementary challenges. Individual infiltrative cells do not depend on angiogenesis.15 On the contrary, it has been suggested that, in response to bevacizumab, infiltrative growth, as well as other forms of parenchymal invasion, may even be increased.15,22,54,57

The effect of bevacizumab on drug delivery is also complex.
are thought to contribute to pathology. In this case, interpretation is aided by simpler treatments and well-controlled studies. Although rituximab shows efficacy in MS, the mechanism and sites of action are not known. In particular, because autoimmune B cells must enter the brain from the blood, efficacy does not necessarily reflect attack of targets in the brain.

Trastuzumab and metastatic breast cancer. The challenge of late brain metastases. As use of trastuzumab for Her2-overexpressing breast cancer became widespread, a troubling finding was an apparent increase in brain metastases. The reasons are not known. Brain metastases are typically a late occurrence in breast cancer, and so longer survival itself is relevant. However, its role depends upon a point that is often not explicitly discussed: Brain metastases often occur in parallel with tumor control at other sites. If the patient has responded to antibody therapy in the periphery, what can be the source of the newly-detected brain metastases?

Model 1. New blood-borne tumor. Late brain metastases may represent new blood-borne tumor from outside the brain, perhaps from known sites of apparently stable disease or from undetected, dormant tumor elsewhere. Eventually, this tumor begins to grow and provides a source of new blood-borne metastases.

Model 2. Dormant tumor in the brain. As an alternative scenario, tumor may have entered the brain long before it is detected and remained, undetected, as micro-metastases in a dormant state. As long as the BBB is close to normal, then systemic rituximab has shown efficacy for patients with autoimmune diseases, especially multiple sclerosis (MS), where B cells are thought to contribute to pathology. In this case, interpretation is aided by simpler treatments and well-controlled studies. Although rituximab shows efficacy in MS, the mechanism and sites of action are not known. In particular, because autoimmune B cells must enter the brain from the blood, efficacy does not necessarily reflect attack of targets in the brain.
contrast-enhancing agents, such as gadolinium, would not reveal this tumor and antibody therapeutics would not reach it (Fig. 3B). Once the tumor begins to grow, the BBB might initially be compromised enough for gadolinium, but not antibodies, to extravasate (Fig. 3C). The tumor could thus be detected radiographically, but not treated.

As the tumor continues to grow, the barrier will break down further and antibody may then extravasate (Fig. 3D). Therefore, if the same mAb treatment is continued, it may show efficacy eventually. Actively delivering the antibody across the BBB should allow for an earlier response. If the tumor grows too large before antibody is given, impediments besides the BBB will become increasingly important in blocking distribution of the antibody, even if it does leave the vessel (Fig. 3E).

The ability of systemic antibody to help control brain metastases would thus vary with the tumor's current site (that is, whether or not it was already in the brain) and size, as depicted in Figure 3 as idealized examples. Brain metastases are heterogeneous, even within an individual, and may differ in the time of entry to the brain, susceptibility to the antibody in question and BBB status; moreover, each of these factors can change with time. Even with these caveats, the models compared above can help to interpret clinical findings, especially partial or disappointing responses.

In practice. Several authors have suggested that systemic antibody should be continued after brain metastases are detected in breast cancer patients and evidence of benefit has been reported. Interpretation is complicated by the same factors discussed above: Many studies are small or anecdotal and multiple modalities or agents may be involved. When there is apparent benefit, the key sites are not known. As many authors discuss, benefit from systemic antibody may simply reflect better control of systemic disease; it does not necessarily reflect direct attack of tumor in the brain. In practice, the potential for direct attack is likely to vary among different metastases and to change with time (Fig. 3).

Summary of clinical experience. There is evidence that systemic mAb treatment can benefit patients with brain tumors or other CNS pathology. The nature and site of antibody activity are less clear. The extent to which antibody enters—and acts at—tumor sites within the brain itself is not known. A conservative interpretation is that bevacizumab primarily reduces edema, and rituximab and trastuzumab act primarily on systemic targets. The complexity of tumor therapy, difficulty of direct local measurements, limitations of clinical trials and drawbacks of preclinical models all complicate interpretation of clinical results. Regardless of whether the antibody acted in the brain or elsewhere, an increase in overall or progression-free survival, or simply an improved quality of life, are certain of benefit to brain tumor patients. The goals for the future are, as for all tumors, to increase the benefit and reduce the cost of the therapeutics.

Related topics. The text has emphasized disseminated tumor within the brain, for which systemic delivery of mAbs is especially relevant. The focus has been on the role of the BBB, rather than specific strategies to overcome it, or cases, such as meningeval tumor, where other delivery routes are of special interest. The points raised should help to interpret findings for a variety of delivery strategies and tumor sites.

The Future

The natural evolution of mAb therapy for any tumor at any site is towards redundancy and refinement. Redundancy, in the sense that alternative targets are identified and alternative antibodies are prepared against promising targets, old or new. Refinement, in the sense that the new antibodies can be designed to solve specific problems: to avoid known cross-reactions or to work by means of alternative effector mechanisms. Refinement of another kind will come from combining therapies in more directed ways, in parallel with growing understanding of the underlying mechanisms of tumor growth, susceptibility and resistance. Combined targeting of a GBM tumor mass plus infiltrative tumor or existing brain metastases plus tumor that has not yet reached the brain are obvious examples. Improved clinical trial design will be important for all tumors, as will more predictive preclinical models.

As for mAbs themselves, two parallel approaches are each likely to be fruitful. One is to continue to refine the specificity and modifications of the antibody molecule itself. The other is to synthesize novel agents, using knowledge of antibody structure and function as a guide. Even as novel agents evolve, the whole antibody molecule still has great value. It has a long half-life and can mediate multiple functions, with new functions and uses still being identified. Indeed, the key mechanisms used by even the most successful antibodies in human patients are not yet established.

For all solid tumors, a complementary evolution of both understanding and technology is needed to improve delivery of therapeutics to larger tumor masses. For the brain, where delivery to micro-tumor is an equal challenge, clearer understanding of the nature and role of the BBB, complemented by improved methods for opening or bypassing it when necessary, have been long-standing goals. A third approach has received less attention: In other clinical contexts, sustained antibody synthesis occurs within the CNS. This too should be exploitable for brain tumor patients.

In the long run, how will mAb therapeutics benefit brain tumor patients? We may depend on antibody itself, a fragment or a synthetic alternative; the agent may be delivered passively or actively made within the brain; it may attack brain tumor directly or “only” targets outside the brain (such as blood-borne metastases or tumor vasculature); or even less directly, it may act to simulate or modify an endogenous response. Most likely, each variation will have its role. In planning and interpreting antibody therapy, the BBB has rightly commanded attention, yet its importance is balanced by that of more general problems of access and resistance. Going forward, a matching focus on challenges that are common to other agents, other tumors and other sites will be a complementary way of getting past the BBB.
Acknowledgements

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References


