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Promising approaches to circumvent the blood-brain barrier: progress, pitfalls and clinical prospects in brain cancer

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Abstract

Brain drug delivery is a major challenge for therapy of central nervous system (CNS) diseases. Biochemical modifications of drugs or drug nanocarriers, methods of local delivery, and blood–brain barrier (BBB) disruption with focused ultrasound and microbubbles are promising approaches which enhance transport or bypass the BBB. These approaches are discussed in the context of brain cancer as an example in CNS drug development. Targeting to receptors enabling transport across the BBB offers noninvasive delivery of small molecule and biological cancer therapeutics. Local delivery methods enable high dose delivery while avoiding systemic exposure. BBB disruption with focused ultrasound and microbubbles offers local and noninvasive treatment. Clinical trials show the prospects of these technologies and point to challenges for the future.

The blood–brain barrier (BBB) present in brain capillaries poses a formidable challenge for delivery of therapeutics to treat a wide array of diseases affecting the brain (e.g., primary and secondary brain tumors, neurodegenerative diseases, lysosomal storage disorders, infections) [1–3]. As a consequence of the BBB, drugs which may be otherwise effective cannot be utilized, require invasive delivery methods or have limited efficacy as well as side effects resulting from the use of large dosages.

The BBB phenotype is altered in many CNS diseases (see [4] for an extensive list) which can have important implications for drug delivery. In the context of brain cancer, a proangiogenic environment yields blood vessels with a disorganized endothelium and altered blood flow which can reduce perfusion of affected tumor regions [5,6]. Hyper-permeability of the tumor vasculature is often observed as a result of increased paracellular flux due to loss of tight junctions as well as increased fenestrations and transcytotic vesicles in endothelial cells (ECs) [5,6]. The heterogeneous permeability of the BBB in tumors can reportedly limit drug efficacy [7,8]. A recent study found that the measured concentration of systemically administered capecitabine or lapatinib within intracranial tumors was variable

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which resulted in limited efficacy in many cases in patients with breast-derived metastatic brain cancer [8]. The BBB may remain intact in infiltrating gliomas or micrometastatic tumors, and these sites may be the source of tumor recurrence [2,5]. Additional barriers to effective brain tumor delivery are multidrug resistance imparted by active efflux transporters (AETs) or drug metabolizing enzymes present in brain ECs, and elevated interstitial fluid pressure [9,10]. Given the evidence that suggests the BBB remains an obstacle to treating brain tumors with pharmaceutical agents, development of approaches which circumvent the BBB continues to be an active area of research in cancer therapy.

Biochemical modifications of drug formulations and local delivery methods have been developed to circumvent the BBB by enhancing transport across or bypassing the BBB. A biochemical modification with considerable promise is to target drug conjugates or nanocarriers to receptors which enable receptor-mediated transport (RMT) across the BBB. This strategy has the advantage of being a noninvasive method applicable to small molecules and biologics (e.g., therapeutic peptides/ proteins or nucleic acids), and can distribute drugs throughout the brain tissue [1]. Local delivery methods bypass the BBB by delivering the drug to discrete sites within brain tissue using implantable drug depots or direct infusion [3,11]. Implantable drug depots are placed at the desired therapeutic site (e.g., the tumor resection cavity for adjuvant chemotherapy of brain tumors) and elute drug into the adjacent tissue. Direct infusion into brain tissue can be accomplished by convection-enhanced delivery (CED). Furthermore, local delivery methods and biochemical modifications of drug formulations can be combined in a given strategy of circumventing the BBB (e.g., packaging chemo-therapeutics in nanocarriers and delivering to tumors via CED).

A local and noninvasive strategy for BBB disruption is the use of focused ultrasound (FUS) in conjunction with ultrasound contrast agents (i.e., stabilized microbubbles approved by the US FDA for contrast-enhanced diagnostic ultrasound) [2,12]. When driven to oscillate nonlinearly, circulating ultrasound contrast agents generate mechanical forces that can temporarily increase BBB permeability to small (e.g., chemotherapeutic agents) and large (e.g., proteins) biomolecules [2]. The use of focused ultrasound allows for opening the BBB with a great deal of spatial selectivity and delivering anticancer agents specifically to targeted brain tumors [2,12]. The results from animal studies have been very encouraging and are paving the way for clinical trials.

In this review, we will first summarize the anatomical and physiological features of the BBB which create challenges for drug transport. Next, we will examine promising preclinical work for biochemical and local methods of brain delivery. Clinical prospects in brain cancer will then be reviewed, followed by concluding remarks and future perspective for these drug delivery strategies.

Anatomy & physiology of the blood–brain barrier

The cerebral vasculature is nourished by the carotid and vertebral arteries which transport oxygenated blood (15–20% of total cardiac output) from the heart to the brain [13]. The arteries branch to form arterioles which penetrate into brain tissue and continue branching to

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form capillaries. Capillaries are present throughout brain tissue, and it is at this level that transport of solutes from the blood to the brain parenchyma largely occurs.

The BBB is a selective permeability barrier composed of ECs lining the lumen of brain capillaries (Figure 1A). Diffusion across the BBB is limited by the EC plasma membrane, which lacks fenestrations [14]. Diffusion between brain ECs is severely restricted by tight junction complexes [14]. Tight junction complexes are composed of the integral membrane proteins claudins and occludin (Figure 1A inset). Claudins 3 and 5 are mainly present in brain ECs, and interactions of these molecules bridge the paracellular cleft (Figure 1A). Claudin 5 predominates over Claudin 3 [15], and is critical to restricting permeability of small molecules to <800 Daltons [16]. Claudin 3 is present in BBB tight junctions with its loss being linked to increased BBB permeability in experimental autoimmune encephalomyelitis and brain cancer [17]. Occludin also decreases paracellular permeability, and regulates tight junction structure [18,19]. ZO 1–3 are intracellular binding partners of claudins and occludin which interact with the actin cytoskeleton, and this association is hypothesized to aid in maintaining tight junction stability [14].

Blood-to-brain permeability is further restricted by AET and drug metabolizing enzymes (Figure 1A). AET present on the luminal plasma membrane function to transport xenobiotics in the direction of brain-to-blood via ATP-binding cassette transporters of the Pgp family, MRP, and BCRP (Figure 1A) [20,21]. Xenobiotics, which are recognized by these transporters, are used to treat many different diseases, including cancer, malaria, human immunodeficiency virus, dyspepsia, diarrhea, and others [21,22]. Cytochrome P450 enzymes function to metabolize xenobiotics as well as endogenous substrates [23]. They are present on the plasma membrane as well as various intracellular compartments of brain ECs [24]. CYP1B1 is the predominant detected isoform present in brain microvessels, and is preferentially expressed in brain microvessels than in brain cortex [25].

Intercellular communication between brain ECs, pericytes and astrocytes plays an important role in the function of the BBB. Although the precise mechanisms of communication between these cells are still emerging, signaling molecules secreted from pericytes and astrocytes interact with receptors on the abluminal endothelial surface to promote barrier function and regulate BBB permeability (Table 1). For example, upregulation of tight junction protein expression has been reported for Sonic Hedgehog, FGF-2, and GDNF [26–29]. Decreased BBB permeability has been reported in response to Sonic Hedgehog, Ang-1, FGF-2 and GDNF [26–27,29–30]. APoE4 plays a critical role in maintaining BBB integrity [31], and pericyte deficiency has been shown to increase vesicular transcytosis in brain ECs [32].

These various structural and functional aspects of the BBB result in a number of distinctions with nonbrain capillaries. As illustrated in Figure 1B, brain capillaries lack fenestrations (transcellular pores) on the plasma membrane as well as interendothelial gaps which are present in certain nonbrain capillaries to increase permeability of macromolecules [36]. Tight junctions of brain capillaries severely restrict paracellular transport, more so than tight junctions of ECs in nonbrain capillaries (e.g., heart, lungs) [37]. Low/negligible pinocytosis in brain capillaries further restricts nonspecific transport in comparison to other organs [4,5].

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Pericytes and astrocytes interact with brain ECs to promote barrier functions [32,34,37], whereas in nonbrain capillaries astrocytes are absent and pericyte presence does not generate a highly restrictive barrier.

The majority of transport across the BBB occurs in a selective and heavily regulated fashion, and is governed by carrier-mediated transport (CMT) and RMT.CMT enables movement across cell membranes via facilitated or active transport. Because active transport consumes energy, the high concentration of mitochondria present in brain ECs is hypothesized to be due at least partly to these processes. CMT from blood to brain or brain to blood occurs for molecules related to energy production (glucose, lactate), amino acids, nucleosides and various other ionic molecules (Figure 1A) [21,38]. CMT brain-to-blood efflux also aids clearance of neurotoxic substances, metabolites of brain function, neurotransmitters, as well as xenobiotics by the aforementioned drug efflux transporters [21]. RMT is utilized for the transport of macromolecular substances into brain including nutrients, hormones, growth factors and lipoproteins [14]. The general process involves binding to a cell surface receptor present on the luminal surface of brain ECs, endocytosis into the cell interior, vesicular transport from the luminal to abluminal region of the cell and exocytosis of the vesicle resulting in delivery of the substance to the extravascular space (Figure 1A). Mechanisms of action include clathrin- or caveolae-mediated endocytosis, as well as nonclathrin and noncaveolae-mediated mechanisms [39].

Methods to overcome the BBB for delivery of therapeutics to the brain

The BBB represents a major challenge for delivery of therapeutics to the brain with approximately 98% of small molecules and probably all biologics incapable of blood-to-brain transport by free diffusion [40]. The lack of endothelial fenestrations and pinocytosis, as well as the presence of highly impermeable tight junction complexes restricts trans- and para-cellular transport. In addition, AET shuttle drugs back into the blood and drug metabolizing enzymes degrade drugs prior to reaching the target site.

Biochemical modifications to enhance BBB transport covered here include direct modification of the drug itself, as well as targeting drugs or drug nanocarriers to CMT or RMT systems. Also discussed are local methods of brain delivery and FUS-mediated BBB disruption.

Biochemical modification of drug formulations Direct agent modification & CMT

The purpose of direct agent modification is to alter the chemical structure of a therapeutic molecule to enhance transport across the BBB. Lipidization, structural modifications to enhance stability, and conversion to a prodrug have been explored [41]. These strategies have both strengths and weaknesses (see [41]), and are generally applied to small molecules. Physicochemical parameters such as lipophilicity and size as well as recognition by AET or drug metabolizing enzymes bear consideration when modifying therapeutic agents to enhance BBB permeability. Lipophilicity is inversely related to the hydrogen bonding potential of a molecule in water, and can be adjusted using medicinal chemistry (e.g., by blocking functional groups which form hydrogen bonds) [40]. Adjusting physicochemical parameters (e.g., increasing lipophilicity), may have opposing effects on the outcome (e.g.,

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increasing BBB penetration while also increasing tissue penetration in peripheral tissues), which may add complexity to this approach [40,41].

A drug also may be modified (directly or via coupling to an endogenous substrate) to be recognized and transported across the BBB by CMT [42]. The molecular structure of the drug must mimic that of the endogenous CMT substrate (e.g., sugars, amino acids, nucleosides), should preferably not affect CMT function to avoid possible side effects, and must maintain its pharmacologic activity [40,41]. This approach has been applied to small molecules but not high molecular weight biological molecules [40,41].

Recently, it has been shown that targeting nanocarriers to CMTs improves delivery of the cargo across the BBB. For example, liposomes targeting GLUT1 enhanced transport of daunorubicin across the BBB compared with nontargeted liposomes [43]. In another study, liposomes targeting glutathione transporters (2B3–101) enhanced doxorubicin delivery to the brain extracellular space by 4.8-fold compared with nontargeted liposomal doxorubicin [44]. 2B3–101 has reached clinical trials.

Targeting RMT receptors

Drugs or drug nanocarriers can be designed to target receptors that enable transport across the BBB via transcytosis (Figure 2). In this section, we review key concepts and parameters for design of RMT-targeted drug conjugates and nanocarriers for transport across the BBB, followed by an overview of key and emerging RMT receptors for brain cancer therapy.

The RMT-targeting approach involves coupling a targeting ligand (e.g., antibody or antibody fragment, synthetic peptide, natural ligand) which has affinity for an endocytic cell surface receptor expressed in brain ECs to the drug (targeted drug conjugate) or to a drug-loaded nanocarrier. Generally, binding and clustering of the targeted receptor on the cell surface induces intracellular signaling cascades which mediate invagination and pinching off of the plasma membrane to form membrane-bound vesicles in the cell interior. Intracellular vesicular trafficking results in transport from the luminal to abluminal endothelial plasma membrane. Dissociation of the ligand-receptor complex and exocytosis of the vesicle at the abluminal EC plasma membrane would presumably enable nanomedicines to proceed to therapeutic sites within the brain parenchyma, although the mechanistic insights of these aspects remain under investigation (in the case of macromolecular ligands, see [45] for review). Although beyond the scope of the current review, nanocarriers can also mediate transcytosis via use of surface coatings [46], or cell-penetrating peptides, which enable adsorptive-mediated transcytosis [47].

RMT can be utilized to transport high molecular weight biologics (e.g., therapeutic antibodies, peptides, growth factors, etc.) or nanocarriers which are generally too large to be delivered across the BBB by CMT [47,48]. Targeting RMT receptors may also enable targeting and intracellular delivery to neurons, glia or tumors for biological therapeutics which cannot cross cell membranes via diffusion [49,50]. RMT-targeting may avoid interactions with AET or metabolizing enzymes by utilizing vesicular transport rather than diffusion through the cytosol [22]. Nanocarrier-based RMT also offers the possibility of delivering blood-insoluble drugs, shielding drugs from premature degradation (e.g., proteins,

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nucleic acids) or for triggered-release strategies which utilize features of the disease site or external forces (e.g., heat, sound) to selectively release the drug at the target site [51–53]. Nanocarrier size, charge, surface chemistry and parameters of the targeting ligand (e.g., affinity, valency) may also be utilized to optimize drug delivery end points such as circulation time, biodistribution, uptake into cells and intracellular trafficking (selected examples cited here, [54,55]). In contrast to local delivery methods, intravenously administered drugs, which are delivered to the brain via RMT or other mechanisms, can achieve widespread distribution in the brain. This is advantageous for therapies where disease is also widespread in brain tissue (e.g., lysosomal storage disorders), but may be a limitation for therapy of more localized brain pathologies (e.g., brain cancers). The biodistribution for systemically administered nanomedicines remains predominantly favored toward nonbrain tissues, as generally <1% of the injected dose reaches the brain [51]. Pharmacologic activity at this yield is certainly feasible. Yet, increasing this number could improve the therapeutic window of existing treatments, or open the door for therapeutics with unacceptable toxicity in peripheral tissues. Unlike cell penetrating peptides or BBB-permeabilizing agents, which interact with cells and tissues indiscriminately, targeting RMT receptors allows for the possibility of enhancing selectivity for the brain versus other organs. However, this remains challenging due to expression of RMT receptors in peripheral tissues, and clearance by the reticuloendothelial system.

Characteristics of the target receptor (e.g., expression, epitope accessibility, mechanism of endocytosis, function, response to disease) and targeting ligand are important considerations for brain delivery by RMT. A greater differential in expression of the RMT receptor between brain and peripheral tissue is needed for more selective delivery to brain versus peripheral organs. The endocytic mechanism is also an important consideration, as it affects intracellular transport as well as design parameters of nanocarriers [56]. In the context of nanocarrier size, for example, nanocarriers targeted to RMT receptors associated with clathrin- or caveolae-mediated endocytosis may be constrained by the size of endocytic vesicles formed via these pathways (~100 and ~70 nm, respectively) [56–58]. Clathrin- and caveolae-independent mechanisms (e.g., cell-adhesion molecule-mediated endocytosis, phagocytosis) enable internalization of carriers up to microns in size [54,59]. Regarding the targeting ligand, binding affinity and valency as well as selection of the target epitope can influence binding to cells, intracellular transport and ultimately transcytosis capacity [55,60–62]. Targeting should preferably not alter the function of the RMT receptor [56], as this may lead to toxicity or other side effects. The ligand should also not be immunogenic, and the target epitope should be present in humans to enable clinical translation.

Transferrin receptor

Transferrin receptor (TfR) is perhaps the best characterized RMT receptor [40]. The function of the TfR is to enable the transport of iron into cells by endocytosis of its endogenous ligand, Tf. The TfR is relatively abundant on the BBB and liver, and with regard to CNS pathological expression is overexpressed in brain cancers [63]. TfR internalization occurs via clathrin-mediated endocytosis [63].

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TfR targeting ligands utilized for RMT include the endogenous ligand Tf, full length antibodies recognizing TfR [64,65], single-chain variable fragment (scFv) [66], peptides (CRTIGPSVC [67], T7 [68], THR [69]) and aptamer [70]. The Tf targeting ligand competes with endogenous Tf for TfR binding sites, and this may limit binding efficiency as well as inhibit TfR function. Tf has been used in numerous studies to enable internalization in cancer cells [63], but BBB transcytosis via Tf appears limited [39,71]. Many of the alternative targeting ligands have been designed to target epitopes outside of the Tf binding site. Anti-TfR scFv lacks the Fc antibody domain, limiting potential immunogenicity and clearance by cells of the reticuloendothelial system. Aptamers and peptides typically also lack immunogenicity, and can be manufactured via chemical synthesis which can allow for easier scale-up and less batch-to-batch variability [72]. Aptamers can also be designed to have higher affinity than antibodies. The T7 peptide (HAIYPRH) binds TfR with a similar affinity as Tf, although it does not compete for the Tf binding site [68]. The cyclic peptide CRTIGPSVC interacts with apotransferrin to induce binding of the apo-Tf/CRTIGPSVC complex to TfR [67]. Experiments in mice also provided evidence that CRTIGPSVC is capable of transcytosis *in vivo* [67]. DNA- and RNA-based aptamers designed to target the extracellular domain of TfR demonstrated TfR-specific accumulation in cells without interfering with Tf binding [70]. Aptamer transcytosis was not examined in the study.

A number of studies have elucidated parameters which may be used to optimize targeting for brain delivery (Tables 2 & 3). In the case of TfR, selection of the targeted TfR epitope can affect the biodistribution. Distinct anti-TfR monoclonal antibodies were found to accumulate in brain tissue and have differential biodistribution in mice [64]. Anti-TfR clone 8D3 was found to have higher overall accumulation in brain, while anti-TfR clone R17217 had lower liver accumulation, displaying greater selectivity for brain [64]. The Trump antibody which binds to a TfR epitope present on high but not low grade lymphomas was demonstrated to distinguish malignant cell status [73]. The number of OX-26 antibodies conjugated to polymersomes was optimized to maximize BBB penetration [65]. The coupling method of anti-TfR mAb to nanocarriers also appears important, as covalent conjugation to liposomes enhanced transport across an *in vitro* BBB model in comparison to biotin-streptavidin coupling [74]. Antibody affinity toward TfR affects transcytosis, as high affinity antibodies remain trapped within brain capillaries *in vivo*, while lower affinity antibodies achieve transport from blood to brain parenchyma [61]. Further, high affinity binding of antibody to TfR alters intracellular trafficking away from transcytosis and toward lysosomes [60]. In another study, anti-TfR antibodies which had pH-dependent affinity toward TfR (higher TfR affinity at extracellular pH, lower in endosomes) were transported across a cell culture BBB model, whereas antibodies with pH-independent affinity for TfR remained in intracellular compartments and were degraded [75]. Introducing a ‘binding module’ into a therapeutic protein which enables monovalent binding of TfR was recently shown to enhance transcytosis across the BBB compared with divalent binding [62].

Cancer therapeutics delivered across the BBB via TfR-targeting include epirubicin [83], doxorubicin [84,85], 5-fluorouracil [86], p53 plasmid [87], daunorubicin [43], cisplatin [50] and short hairpin RNA (shRNA) plasmid [88]. One study aimed at enhancing BBB transport by designing liposomes to both inhibit AET activity and target TfR via display of Tf and

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tamoxifen (an inhibitor of AET). These liposomes were shown to enhance transport of epirubicin across brain EC monolayers, as well as uptake by glioma cells and inhibition of glioma spheroid volume *in vitro* [83]. Compared to controls, the TfR-targeted liposomal epirubicin increased the survival time of rats burdened with intracranial glioma tumors [83]. Another study demonstrated that Tf-liposomes loaded with doxorubicin decreased expression of Pgp efflux pumps, which enhanced BBB penetration of doxorubicin [84]. The liposomes were targeted to TfR to enhance BBB penetration, and to the folate receptor to target cancer cells [84]. Enhanced delivery of doxorubicin was demonstrated via targeting both TfR and folate receptor over single-targeted liposomes [84]. A separate study adopted the novel approach of combining RMT- and CMT-based targeting for selective delivery of liposomal daunorubicin across the BBB to malignant gliomas. These liposomes inhibited glioma cell proliferation and improved survival over single-targeted liposomal daunorubicin [43].

Another important development has been proof-of-concept studies of gene therapies for brain cancer. RNA delivery to brain cancer cells was demonstrated *in vivo* using TfR-targeted, PEGylated liposomes [88]. The authors utilized a DNA-based plasmid encoding shRNA to knockdown luciferase reporter in intracranial glioma [88]. The same group utilized avidin-biotin coupling to develop an antibody-small interfering ribonucleic acid (siRNA) conjugate for brain cancer therapy [89]. Another gene therapy that has now reached clinical trials is SGT-53, a cationic liposome modified with an anti-TfR scFv and loaded with DNA plasmid encoding for the *p53* tumor suppressor (see section: ‘Clinical trials in brain cancer’) [87]. Gene therapy for brain cancer may reduce toxicity through the use of promoters which selectively activate transcription in tumor rather than healthy tissue, or by sensitizing drug resistant tumor cells to chemotherapy [90].

LRP1

LRP1 is a multifunctional receptor of the LDLR family which mediates the transport of diverse ligands including growth factors, protease inhibitor complexes and extracellular matrix proteins [91]. LRP1 regulates response to inflammation in pathological conditions [91]. In cancer cells, LRP1 supports cancer cell invasion by functioning as a regulator of cytoskeleton organization and adhesive complex turnover [92]. LRP1 expression is present in brain ECs and cancer cells including malignant glioma [91]. LRP1 internalization occurs via clathrin-mediated endocytosis, although caveolae-mediated endocytosis has also been reported [88,91]. There is emerging evidence for LRP1 as a therapeutic target due to its role in cancer cell invasion [93].

Targeting ligands for LRP1 include natural ligands (p97, RAP, lactoferrin) and synthetic peptides (angiopep-2, [71], and ApoE3 analog, [76]). Synthetic peptides offer advantages as targeting ligands due to their low potential immunogenicity/toxicity and high specificity. Table 2 provides a summary of peptides targeting RMT receptors from the literature. p97, RAP, lactoferrin and angiopep-2 have been reported to enable targeting and transcytosis of brain ECs, with angiopep-2 and p97 enabling greater transcytosis than lactoferrin and transferrin [94,95]. Competition with endogenous ligand may be minimal for p97 due to its low endogenous serum level [96]. Angiopep-2 binds to an LRP1 epitope which appears to

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overlap with alpha-2-macroglobulin, but this does not appear to hinder angiopep-2 BBB transport [94,97]. The ApoE3-derived peptide demonstrates targeting and transcytosis *in vitro*, but loses this capacity *in vivo* or in presence of serum [98]. Interestingly, angiopep-2 conjugated to liposomes did not show apparent brain targeting, suggesting that design parameters (e.g., nanocarrier size, coupling chemistry, linker length and valency, among others) may significantly affect binding of angiopep-2 [98]. However, nanocarriers modified with angiopep-2 have demonstrated targeting capacity in other studies [49,99–100]. The valency of angiopep-2 was optimized to enhance brain accumulation of dendrimers, with four angiopep-2 ligands demonstrating the highest accumulation in brain [101].

Brain cancer therapeutics delivered via LRP1-targeted drug conjugates include paclitaxel [96,99,102], adriamycin [96] and tumor necrosis factor-related, apoptosis-inducing ligand gene [49]. A drug conjugate composed of angiopep-2 and paclitaxel (Ang-1005) demonstrated significant improvement in brain uptake over unconjugated paclitaxel in breast-derived metastatic brain cancer [103]. Ang-1005 avoided paclitaxel efflux by Pgp, as evidenced by similar brain uptake in wild-type and Pgp knockout mice [104]. Angiopep-2 conjugated to doxorubicin or etoposide was also shown to increase brain penetration over unconjugated drug and avoid Pgp-mediated efflux [97]. Drug conjugates composed of human p97 and adriamycin or paclitaxel enhanced brain accumulation by tenfold over unconjugated counterparts with p97-adriamycin improving survival time over free adriamycin in mice bearing intracranial gliomas or breast-derived metastatic tumors [96]. Recently, angiopep-2 conjugated to an antibody against HER-2-targeted brain ECs, maintained HER-2-dependent antiproliferative activity against breast cancer cells and improved survival over unconjugated anti-HER-2 in breast-derived intracranial tumors [105]. Development of Ang-1005 has reached clinical trials.

With regard to LRP1-targeted drug nanocarriers, polymeric nanoparticles modified with angiopep-2 enhanced paclitaxel delivery and antitumor activity over free paclitaxel and nontargeted polymeric nanoparticles in tumor spheroids and intracranial glioma tumors [99]. Previous studies have demonstrated that targeting and uptake of angiopep-2 polymeric nanoparticles was governed by LRP1-dependent clathrin- and caveolae-mediated endocytosis [106,107]. Dendrimers modified with angiopep-2 were shown to enhance cytotoxicity to glioma cells *in vitro* and in intracranial glioma tumors, as well as improve overall survival compared with temozolomide (TMZ) [49]. Cationic PEGylated liposomes modified with angiopep-2 and a tumor homing peptide targeting the neuropilin-1 receptor and co-loaded with docetaxel and siRNA against VEGF demonstrated synergistic inhibition of tumor growth in subcutaneous glioma tumors without apparent immunogenicity [108].

Insulin receptor

Insulin receptor (IR) functions in cell metabolism by mediating the transport of insulin, a hormone regulating glucose catabolism, into cells. IR is present on brain ECs and brain cancer cells, and is internalized through multiple endocytic pathways [56]. IR targeting ligands include monoclonal antibody 83–14 against human IR [109]. Blood-to-brain transcytosis of antibody 83–14 has been reported in nonhuman primates [110], and a fully humanized IR antibody has been developed [111]. One potential disadvantage of targeting

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IR is inhibition of insulin function. Design considerations such as selection of the target epitope and size of the drug delivery system may help minimize competition with endogenous insulin or steric hindrance of the insulin binding domain, respectively.

Therapeutic compounds for brain cancer targeted via IR include siRNA against EGFR [112]. PEGylated immunoliposomes displaying antibody 83–14 delivered a DNA-based plasmid into glioma cell cultures [112]. Delivery to nearly all cells with considerable reduction in EGFR activity was reported [112]. A number of anti-IR fusions have been created for CNS disorders other than brain cancer (e.g., Alzheimer's disease, lysosomal storage disorders [1]), and polymersomes conjugated with antibody 83–14 target brain ECs *in vitro* [113].

Low density lipoprotein receptor

Low density lipoprotein receptor (LDLR) functions in transport and clearance of lipoproteins, and is upregulated in brain ECs, liver and cancer cells with likely ubiquitous lower levels of expression in other tissues. Expression of LDLR on the BBB depends on lipid need [114], suggesting that dietary parameters such as body rhythms [39], may possibly be exploited to increase the unbound LDLR receptor pool. LDLR appears to be internalized via multiple pathways dependent on the cell status. Caveolae-mediated endocytosis is utilized for LDLR internalization in nonproliferating cells and clathrin-mediated endocytosis in proliferating cells [39].

Targeting ligands of LDLR include natural ligands (ApoE, [115]) and peptides (peptide-22, COG133) with transcytosis across the BBB being reported. ApoE-targeted albumin nanoparticles cross the BBB and reach neurons in mice [115]. COG133 has been shown to transport across the BBB, but lost targeting specificity when conjugated to liposomes [98]. Peptide-22 is a cyclic 8-mer peptide which was optimized to bind LDLR with high affinity and without competition of endogenous LDL [80]. With regard to delivery of therapeutics for brain cancer, peptide-22 modified polymeric nanoparticles loaded with paclitaxel were trafficked across an *in vitro* BBB model through clathrin- and caveolae-mediated endocytosis [116]. Peptide-22 increased transport across the BBB and glioma cell death [116].

Leptin receptor

The leptin receptor (ObR) functions in regulation of energy storage by transport of the hormone leptin which inhibits hunger. A short form of the ObR receptor is enriched in cerebral microvessels and suggested to mediate leptin transport across the BBB [117], while a long form ObR appears higher in adipose and liver tissues [118], suggesting that targeting the short form ObR may be advantageous for brain delivery. Decreased BBB transport of leptin in mice lacking the short form ObR supports this notion [119]. ObR is overexpressed in hormone-dependent cancers, and has been proposed as a therapeutic target [120]. ObR internalization occurs via clathrin-mediated endocytosis [121,122] and perhaps also nonclathrin-/noncaveolae-mediated endocytosis [122]. One potential disadvantage of the ObR system is that it is thought to be impaired in obese patients [39,123,124].

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Targeting ligands for ObR include its natural ligand (leptin [119]) as well as leptin-derived peptides (Table 2). Leptin, Leptin30 and G21 have been reported to transport across BBB models *in vitro* and/or *in vivo*. Leptin has been suggested to transport across the BBB via both an ObR- dependent and an ObR-independent process [78]. G21 and leptin30 peptides are derived from leptin residues demonstrated to enable transport across the BBB (leptin residues 1–33 and 61–90, respectively [77]). G21 is a shortened form of leptin1–33 which displayed higher BBB transport than peptide composed of leptin residues 1–33 [77]. Leptin30 is composed of leptin residues 61–90, and leptin70–89 is a truncated form of leptin30. Leptin30 nanoparticles were transported across BBB models *in vitro* and *in vivo* [125]. Uptake of the leptin30 nanoparticles appeared mediated by multiple endocytic pathways with leptin30 inducing uptake via clathrin-mediated endocytosis, and the nanoparticles inducing uptake via caveolae-mediated endocytosis and macropinocytosis [125]. G21-coupled nanoparticles crossed the BBB after intravenous administration and localization in brain tissue was confirmed by electron microscopy [126]. It is unclear whether BBB transcytosis occurs in the case of the leptin70–89 peptide. Leptin70–89 coupled to PEGylated liposomes improved internalization in brain ECs, which was suggested to occur mainly via macropinocytosis [78].

Nicotinic acetylcholine receptor

Nicotinic acetylcholine receptors (nAChRs) enable the transport of the neurotransmitter acetylcholine into cells [127]. nAChRs are expressed on the BBB, astrocytes, neurons and peripheral tissues [127]. nAChRs are exploited by pathogens and nicotine to reach the brain. Targeting ligands for nAChRs are peptides derived from RVG29 or CDX, and mediate BBB transport. The RVG29 peptide has not been used for brain cancer therapy, but an RVG29-siRNA conjugate was demonstrated to transport across the BBB and into neurons after intravenous injection [128]. RVG29 has also been used to enhance brain targeting of dendrimers [81]. CDX has been utilized to deliver paclitaxel for brain cancer therapy. Paclitaxel-loaded micelles targeted to nAChRs via CDX were shown to accumulate in brain, inhibit tumor growth and prolong survival of mice with intracranial glioma tumors [82].

Local administration

An alternative approach which bypasses the BBB is local administration of therapeutics to brain tumors via an implanted drug depot or convection-enhanced delivery. Both approaches avoid the myriad of toxicities associated with systemic administration, thus enabling the use of higher drug dosages. Each delivery strategy has advantages and disadvantages, which will be discussed in the following paragraphs.

Implantable devices

Implantable drug depots are installed during surgical intervention and elute drug into the resected margins in order to combat local tumor recurrence. Gliadel® (NobelPharma in Tokyo, Japan), which is a biodegradable polymeric wafer loaded with carmustine (BCNU), was one of the first implantable drug depots to be evaluated clinically against primary brain tumors [129]. In placebo-controlled clinical studies, the adjuvant use of Gliadel alone or in combination with radiotherapy extended the median survival time of patients with primary

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or recurring glioblastoma by 2–3 months [130,131]. The modest impact Gliadel has on patient survival suggests that the malignant cells may have limited exposure (either in time or dose) to eluted drug. This may result from poor diffusion of eluted BCNU in and through the tumor margin [132,133] and/ or insufficient drug loaded into the resected cavity. The first shortcoming can be resolved by replacing BCNU with a drug that penetrates deeper into brain tissue (i.e., TMZ). A number of novel implantable devices designed for loading and releasing TMZ or other lipophilic drugs that are potent against brain cancers are being developed [134–136]. The second shortcoming can be addressed by supplementing the BCNU wafers with another anticancer agent. For example, a clinical trial has shown that the median survival of glioblastoma patients treated with surgery, radiotherapy and Gliadel can be increased by 8 months with the addition of TMZ administered orally [137]. The most effective implantable device will combine controlled and predictable release kinetics with a drug that can diffuse away from the source, ultimately achieving a volume of distribution that closely matches the unresected tumor volume. Although implantable depots have numerous appealing aspects, their effectiveness for intracranial tumor therapy may be limited due to an inability to replenish the reservoir, change its location or modulate drug release kinetics without surgical intervention.

Convection-enhanced delivery

Studies have shown that macromolecules and bioactive agents administered locally in the brain have a limited penetration depth due to poor diffusion [133,138,139]. This is a major obstacle to effective pharmaceutical intervention of brain tumors and other neurological diseases. Convection-enhanced delivery (CED) presents a unique opportunity to overcome this hurdle by enhancing the distribution, and subsequently, the efficacy of administered drugs in the brain [140–142]. In this strategy, a solution containing the molecule or compound of interest is infused through a cannula that has been inserted into the tissue. By maintaining a pressure differential between the cannula tip and the surrounding tissue, the administered drug is transported into the tissue via convective flow [143]. Deeper tissue penetration can be achieved via CED, resulting in a large volume of distribution (V_d) relative to the infused volume [144]. Unlike diffusive transport, CED does not rely upon concentration gradients, making it possible to distribute macromolecules more uniformly throughout the target tissue (Figure 3). Studies have shown that the design of the delivery apparatus and choice of infusion parameters, including cannula diameter, insertion speed and flow rates, is critical to avoiding reflux, controlling the V_d, and minimizing tissue damage [143,145–147]. Incorporating imaging contrast material, such as radiotracers, iodinated compounds or paramagnetic materials, into the infusion volume (V_i) enables the use of diagnostic imaging platforms for real-time monitoring of CED and approximating the V_d/V_i ratio [148–153]. Given the sensitivity of the organ to chemical (i.e., pharmaceutics) and mechanical stresses (i.e., pressure), keeping tight control of the V_d limits drug exposure to healthy brain tissue and protects neurological function. Keeping these factors in mind, CED has been used to enhance the delivery of a broad spectrum of chemotherapeutic agents [154–159] and proteins [160–162] with minimal neurotoxicity.

CED has also demonstrated the capacity to deliver nanocarriers and viral vectors loaded with anticancer drugs or genes to targeted brain tumors [3,163–172]. In several studies,

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packaging therapeutics within nanocarriers administered via CED significantly increased the residence time of the drug within brain parenchyma, which positively impacted drug efficacy against established tumors and improved survival rates in tumor-burdened rodents [173–176]. It is important to note that care must be taken in nanocarrier and viral vector design in order to optimize tissue penetration and minimize clearance [177,178]. Toward this end, polymeric nanoparticles have been designed with the appropriate size and surface chemistry to increase penetration into brain parenchyma and improve drug distribution [179,180]. Overall, combining CED with nanocarriers or viral vectors for therapeutic intervention in the brain shows tremendous promise.

FUS-mediated BBB disruption

A compelling strategy for improving drug and gene delivery to brain tissue is the use of transcranial FUS to open the BBB temporarily [181–183]. While studies have shown that FUS alone can disrupt the BBB, this bioeffect was often accompanied by necrosis of surrounding tissue (i.e., lesion) or intracerebral hemorrhage [184,185]. A study published by Vykhodtseva *et al*. in 1995 reported that FUS-mediated BBB disruption was possible without tissue damage or hemorrhage [186]. The authors detected subharmonic emissions during sonication, which suggested that oscillating microbubbles (i.e., acoustic cavitation) played a role in opening the BBB. Subsequent studies have shown that oscillating microbubbles driven by FUS can dramatically alter the morphology of intracranial blood vessel walls (Figure 4), including increased fenestration, induction of vesicular transcytosis and opening of tight junctions [187–189]. These alterations increase the permeability of the BBB temporarily, leading to an increase in the passive diffusion or active transport of imaging contrast materials and therapeutic agents [2]. However, gas bubbles are not prevalent *in vivo*, and consequently, the pressure threshold for FUS-mediated BBB disruption was considerably high. In a seminal study published in 2001, Hynynen *et al*. reported that the pressure required for FUS-mediated opening of the BBB could be reduced by two orders of magnitude by injecting ultrasound contrast agents (i.e., microbubbles coated with surface active agents) before sonication [190]. Furthermore, the authors used contrast-enhanced magnetic resonance (MR) imaging for the first time to confirm FUS-mediated disruption of the BBB. MRI-based assessment of BBB permeability post-FUS exposure was a significant improvement in experimental design as the approach made animal sacrifice unnecessary and enabled studies on the safety and effectiveness of FUS-mediated BBB disruption [191,192].

Since the 2001 paper, numerous studies have explored the relationship between FUS-mediated BBB opening with acoustic parameters [193–197], type and concentration of administered ultrasound contrast agent [196,197], and acoustic cavitation activity [198–201]. While the BBB can be permeabilized using a broad range of parameters, there are some noticeable trends in the published results. Ultrasound attenuation in the skull is inversely proportional to frequency [202]; thus, lower frequencies are more ideal for transmitting ultrasound into the brain. While megahertz frequencies can be used for small animal experiments [203], submegahertz frequencies are more applicable for safely and reversibly opening the BBB in large animals with FUS (i.e., rabbits, primates, etc.) [200,204–206]. Transcranial phased arrays that operate at submegahertz frequencies and

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enable steering of the ultrasound field through tissue are being developed for clinical applications [207–210]. As mentioned previously, the use of ultrasound contrast agents dramatically reduces the pressure required for cavitation-mediated BBB disruption. It is important to note, however, that the likelihood of tissue damage and hemorrhage is directly related to the acoustic pressure, pulse length and total exposure time [194,211–212]. Studies in which the cavitation activity was critically evaluated report that the BBB can be opened safely and temporarily by bubbles that undergo stable nonlinear oscillations, whereas bubbles that collapse inertially result in irreparable damage to the vessel wall [198–199,213]. Results from recent studies suggest that the pressure required for BBB opening may have an inverse dependence on microbubble size [214–216], but this relationship has only been examined at megahertz ultrasound frequencies. Collectively, the studies point toward the use of phased arrays operating at submegahertz frequencies transmitting microsecond pulses at pressures below the inertial cavitation threshold for BBB opening in the clinical setting.

FUS combined with ultrasound contrast agents has been used to increase the BBB permeability to a broad spectrum of therapeutic agents, including small molecule cytotoxic compounds [217–221] and proteins [222–224]. The delivery strategy can also be employed to improve the accumulation of viral vectors [225–227] and nanoparticles [228–234] in the brain, enabling gene therapy of brain disorders and novel theranostic applications. Bioactive macromolecules such as antibodies, neurotrophic factors or siRNA delivered to brain tissue via FUS-mediated BBB disruption remained functional, binding with a targeted antigen [235,236], triggering neuroregeneration [237] or inhibiting expression of a targeted protein [238], respectively. In the case of brain tumors, FUS-induced opening of the BBB has led to a greater accumulation of anticancer agents, which resulted in a strong therapeutic effect [239–241]. The efficacy of potent anticancer agents against brain tumors can be enhanced further with multiple openings of the BBB coupled with administration of the agent [24,242,243]. While results from studies of FUS-enhanced drug transport across the BBB in brain tumors have been encouraging, there are key aspects of the technique that need further development and investigation to enable clinical translation. First, the relationship between applied pressure and the size of FUS-induced openings in the BBB has been explored at megahertz frequencies [244,245] but needs to be studied at the clinically relevant submegahertz frequencies. Next, the spatial distribution of therapeutic agents and drug-loaded nanoparticles after crossing the BBB as a function of physicochemical properties (i.e., molecular weight, size and surface chemistry) needs to be investigated. Overall, the ability to open the BBB reversibly with focused ultrasound in a noninvasive and targeted manner is extremely appealing and has the potential to positively impact the treatment of a variety of brain diseases.

Clinical trials in brain cancer

Brain cancers, particularly malignant gliomas and metastatic brain cancer, are associated with poor prognosis and effective therapies are needed [5,246]. Malignant gliomas are highly infiltrative brain tumors arising from glial cell dysfunction, have an annual incidence of approximately 5 per 100,000 persons and less than 2 years median survival for glioblastoma and 3–7 years for anaplastic gliomas [247]. Diffuse and aggressive expansion

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of malignant gliomas in brain tissues makes complete surgical resection very difficult or impossible. Tumor debulking surgery followed by adjuvant TMZ chemotherapy and/or radiation therapy is the current standard of care [51]. Metastatic brain cancers are tumors which develop secondarily in brain after migrating from the primary site of a nonbrain tumor, primarily lung, breast and skin [5]. The incidence of brain metastasis is approximately 200,000 cases per year in the USA, and although metastatic brain tumors can be completely resected due to presence of clearly defined tumor masses, they can sometimes be located near critical structures which prohibits resection [5]. Recurrence after resection can occur due to the presence of micrometastatic deposits that are below the detection threshold of current imaging techniques [5]. Clinical translation of therapies which circumvent the BBB are of the utmost importance for treatment of malignant gliomas and metastatic brain tumors. BBB permeability is heterogeneous in the tumor area. Areas of infiltrating tumor and smaller tumor masses may be particularly difficult to treat because the BBB may remain intact [2,5]. Many chemotherapeutics cannot cross the BBB, (e.g., doxorubicin, paclitaxel, cisplatin, irinotecan and methotrexate [248]) and are substrates for AET and/or metabolizing enzymes [20,22,23,249], with some or all of these mechanisms restricting their potential efficacy. For example, paclitaxel has been ineffective in a previous clinical trial of recurrent malignant glioma [250].

A drug conjugate designed to enhance BBB permeability of paclitaxel has reached clinical trials for brain cancer (Table 4) [251–253]. Ang-1005 (also named GRN-1005) is a drug conjugate of paclitaxel and the RMT ligand angiopep-2 which targets LRP1. A Phase I study of Ang-1005 in patients with advanced solid tumors and metastases demonstrated that the drug was well tolerated with efficacy in metastatic brain tumors, and a maximum tolerated dose of 650 mg/m^2 [254]. A Phase I study of Ang-1005 in patients with recurrent malignant glioma determined a maximum tolerated dose of 650 mg/m^2, similar toxicity to paclitaxel and lack of immunogenicity [255]. Pharmacokinetic studies and analysis of tumor resections showed that Ang-1005 remained largely intact in blood plasma during the infusion, and achieved intratumoral concentrations of free paclitaxel sufficient for cytotoxicity in all tumor samples [255]. Patient recruitment has begun for Phase II studies of Ang-1005 in patients with recurrent high-grade glioma [252], breast-derived metastatic brain cancer alone or in combination with trastuzumab [251] and recurrent breast-derived metastatic brain cancer [253].

To our knowledge, only a few nanomedicines utilizing nanocarrier-based RMT-targeting have reached clinical trials for brain tumors (Table 4) [256–258]. PEGylated liposomal doxorubicin without RMT-targeting has been evaluated in Phase I studies in patients with malignant glioma, and was well tolerated but did not demonstrate improvement in progression free or overall survival compared with the standard of care [265]. Analysis of preclinical and clinical data suggests that in general ligand-targeted nanocarriers have proven to be safe and effective in preclinical cancer models [266]. In nonbrain tumors the effect of ligand-targeting on nanocarrier extravasation is secondary to passive accumulation into tumors via the enhanced permeability and retention effect [266,267]. Targeting nanocarriers to RMTs may play a more important role for brain cancer, with transcytosis allowing penetration where the BBB remains intact or has low permeability. RMT may also provide a means to limit exposure to AET by intracellular vesicular transport rather than

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diffusion through the cytosol [22,266]. SGT-53 is a nanocarrier composed of cationic liposomes encapsulating plasmid of *p53* tumor suppressor, and displaying scFv-targeting TfR. First in man studies in patients with non-CNS solid tumors were concluded, and reported restoration of p53 function with lack of major side effects [268]. Dose-dependent accumulation of the p53 transgene was observed in metastatic lesions, but not in normal skin tissue, supporting targeted delivery to the tumor site [268]. Patient recruiting for a Phase II clinical trial of SGT-53 in combination with TMZ has begun for patients with recurrent malignant glioma [258]. The end points of the trial include tumor accumulation of SGT-53, induction of apoptosis in tumor cells, 6-month progression free survival, overall survival, antitumor activity and safety [258]. 2B3–101 is a nanocarrier composed of PEGylated liposomal doxorubicin displaying glutathione as a targeting ligand for glutathione transporters (i.e., CMT-based targeting) [44]. Phase I/II clinical trials have been completed for treatment of patients with solid tumors and metastatic brain cancer or malignant recurrent glioma [256], and patient recruiting is underway for patients with breast cancer and leptomeningeal metastasis [257].

A number of Phase I–III clinical trials utilizing CED of immunotoxins or chemotherapeutics in patients with malignant glioma or metastatic brain cancer have been completed (see [269] for review). CED-based therapy remains promising but to date results have not greatly improved overall survival over the current standard of care, and mixed results for a given therapy have been observed [269]. For example, CED of Tf-CRM107 produced complete or partial reduction in tumor size in Phase I/II studies of patients with recurrent primary glioma or metastatic tumors [270]. However, a Phase III study was discontinued prior to completion due to lack of efficacy [269]. The IL-13R-targeted immunotoxin cintredekin besudotox demonstrated an adequate safety profile in early Phase studies [271], but did not demonstrate improved efficacy in comparison to gliadel wafers in a Phase III study (PRECISE trial [272]). Technical concerns such as infusate backflow and catheter positioning, and selection of infusion parameters have been suggested as potential causes of ineffective tumor delivery or accumulation in nontarget brain tissue [273,274]. Retrospective analysis of the PRECISE trial supported the use of computer modeling software as well as alternative catheter designs and infusion parameters to aid CED treatment [274]. Intraoperative imaging is also under development as a solution [142,275]. Current clinical trials appear aimed at adapting these recommendations as well as utilizing novel therapeutic approaches (Table 4) [259–263]. For example, a Phase I trial of CED using image-assisted delivery of liposomal irinotecan is currently recruiting patients with recurrent high-grade glioma [259]. A Phase 0 trial to evaluate CED of topotecan to tumors utilizing image guidance for monitoring of infusion with the Cleveland multiport catheter is recruiting patients with high-grade glioma [260]. A Phase I trial of CED of carboplatin has begun recruitment of patients with recurrent or progressive glioblastoma which may use software in certain patients to predict infusate distribution [261]. Phase I trials utilizing a novel drug conjugate targeting both wild-type EGFR and mutant EGFRvIII (DC27-IT [262]) or oncolytic virus (PVS-RIPO [263]) are also underway. D2C7-IT utilizes a single chain disulphide stabilized variable fragment (scdsFv) of the bivalent anti-EGFR/EGFRvIII antibody D2C7 as a targeting ligand which recognizes both the wild-type and mutant forms of the EGFR, and is fused to domains II and III of pseudomonas exotoxin A [276]. PVS-

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RIPO is a poliovirus chimera which displays potent and preferential cytotoxicity for CNS cancer cells [277], and testing in patients with recurrent glioma is planned [263].

An early phase clinical trial is underway for BBB disruption with FUS. A pilot study using MR-guided FUS to enhance accumulation of doxorubicin in brain tumors and adjacent tissue has recently started patient recruitment to establish safety in humans (Table 4) [264].

Conclusion

The BBB creates challenges for direct blood-to-brain transport of therapeutics. Biochemical modifications of drug formulations, local delivery methods, and FUS-mediated BBB disruption have the potential to improve therapy of CNS disorders by circumventing the BBB. Directly modifying the chemical structure of drugs to enhance intravenous drug transport across the BBB, or modifying drugs to become substrates for CMT remains a viable option only for select small molecules. However, some recent studies have demonstrated that targeting nanocarriers to CMT can enhance BBB transport of chemotherapeutics. Targeting drugs or drug nanocarriers to receptors enabling RMT enhances intravenous drug transport across the BBB of small molecule and biological therapeutics, enabling therapeutic drug concentrations to be achieved for brain cancer therapy in preclinical models. Although the fraction of the injected dose reaching brain via RMT remains low, ongoing development of nanomedicines designed to enhance the transcytosis capacity of RMT receptors may improve upon this aspect. Designing nanomedicines to target receptors enabling RMT may also aid in evading mechanisms of drug resistance by enabling intracellular vesicular transport rather than diffusion. Local delivery using implantable drug depots or CED provides a means to bypass the BBB, delivers relatively large doses to brain tissue and avoids systemic exposure. Implantable depots capable of triggered drug release will enable finer control over pharmacokinetics and possibly improve clinical outcomes. Incorporating imaging agents into infusions for real-time monitoring of CED, as well as combining CED with nanocarriers or viral vectors for therapeutic interventions are promising approaches being implemented to improve CED-based therapies. FUS combined with microbubbles presents a unique noninvasive strategy to open the BBB temporarily in a targeted manner. Phased arrays designed to improve ultrasound transmission through the skull and enable steering of the transmitted waves have been developed in clinical trials for functional neurosurgery, and recently have reached clinical trials for BBB disruption using FUS.

Drug conjugates and nanocarriers aimed at improving effects of chemotherapeutics via RMT or CMT targeting are currently in early-Phase clinical studies for malignant glioma and/or metastatic brain cancers. Early-Phase clinical trials of CED-based therapies for malignant glioma are using imaging methods to monitor drug distribution, as well as testing novel therapeutics to enhance tumor targeting and/or toxicity. An early-Phase clinical trial is also underway for MRgFUS-targeted delivery of doxorubicin in patients with malignant glioma. Further development of these methods appears promising to increase the efficacy or broaden the therapeutic window of existing therapies, or allow the use of drugs which would otherwise be ineffective, opening the door for improved therapy of brain cancer and other diseases of the CNS.

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Future perspective

Improved brain drug delivery may considerably advance treatment of CNS diseases in the next decade. In tandem with these approaches, development of *in vitro* models, which recreate *in vivo* pathology will be critical to screening promising therapies. A contemporary problem in drug development is that drugs which ameliorate disease in cell culture or animal models fail in clinical trials due to a suboptimal relationship to disease in humans. In the case of RMT-targeted nanomedicines and BBB disruption with FUS, *in vitro* cell culture models that recreate the permeability of the *in vivo* BBB in physiological and pathophysiological settings will enable more accurate assessments of drug penetration, as well as provide better insight into success or failure of a given therapy.

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Key terms

Blood-brain barrier	The brain capillary endothelium which restricts and regulates transport between blood and brain
Local delivery	Delivery of pharmaceutical or imaging agents directly into brain tissue
Receptor-mediated transport	Movement into cells and subsequent trafficking within or across cells in membrane-bound vesicles which is enabled by binding to cell surface receptors
Blood-brain barrier disruption	A method/technology which enhances therapeutic transport from blood to brain by increasing permeability of the blood-brain barrier

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Executive summary

Anatomy & physiology of the blood–brain barrier

- The blood–brain barrier (BBB) selectively regulates transport of substances from blood to brain.
- Characteristics of brain endothelial cells (ECs) which contribute to BBB function are tight-junction transmembrane complexes, low pinocytosis, active efflux transporters and drug metabolizing enzymes.
- Transport across the BBB for most substances requires utilization of carrier-mediated transport and receptor-mediated transport systems.
- Intercellular communication between brain ECs, astrocytes and pericytes plays an important role in regulation of BBB permeability.
- Brain capillaries are distinct from nonbrain capillaries in structure and physiology which enables the function of the BBB.

Methods to overcome the BBB for delivery of therapeutics to the brain

- Biochemical modifications of drug formulations
  - Direct agent modification & carrier-mediated transport
    - Direct agent modification involves altering the chemical structure of the therapeutic molecule to enhance BBB penetration.
    - Various strategies have been explored, yet remain limited to relatively small molecules.
    - Modifying molecules to be substrates for CMT and/or avoid efflux pumps/metabolizing enzymes, and maintain activity for the therapeutic target can be complex.
    - 2B3-101 is an intriguing nanocarrier which targets a CMT and has reached clinical trials.
  - Receptor-mediated transport
    - Receptor-mediated transport (RMT) involves targeting a drug or drug nanocarrier to an endocytic cell surface receptor which enables vesicular transport across brain ECs.
    - RMT offers several potential advantages, and is applicable to biologics and nanocarriers which are generally too large to utilize CMT.
    - RMT remains limited, however, by a low percentage of the total injected dose reaching brain.

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- Characteristics of the target receptor, nanocarrier/drug conjugate and targeting ligand are important considerations for development of RMT-based strategies.
- TfR and LRP1 are well established RMT which are being applied to brain cancer.
- Studies of RMT receptor targeting illustrate the capability to optimize delivery across the BBB by modifying parameters of the ligand and/or nanocarrier or by exploiting features of the target receptor.
- IR, LDLR, ObR and nAChR are less explored in the context of brain cancer, yet may merit closer attention as they possess intriguing biological features and/or enhanced BBB penetration of therapeutics in preclinical studies.

• Local administration
  - Implantable devices
    - Implantable drug depots are installed during surgical intervention and elute drug into the resected margins in order to combat local tumor recurrence.
    - The most effective implantable device will combine controlled and predictable release kinetics with a drug that can diffuse away from the source, ultimately achieving a volume of distribution that closely matches the unresected tumor volume.
  - Convection-enhanced delivery
    - Convection-enhanced delivery (CED) presents a unique opportunity to enhance the distribution, and subsequently, the efficacy of administered drugs in the brain by convective flow.
    - Incorporating imaging agents into infusions is promising to improve CED by enabling real-time monitoring of drug distribution.
    - Combining CED with nanocarriers or viral vectors for therapeutic intervention in the brain shows tremendous promise.
  - Focused ultrasound-mediated BBB disruption
    - Focused ultrasound (FUS) transmitted through the skull can disrupt the BBB temporarily via stresses generated by nonlinearly oscillating microbubbles (i.e., acoustic cavitation).

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- BBB disruption is noninvasive and site-specific and can be employed to increase the BBB permeability to a broad spectrum of therapeutics administered systemically, including cytotoxic compounds, nanoparticles and viral vectors.
- Contrast-enhanced MRI can be used for real-time monitoring of FUS-mediated BBB disruption.

Clinical trials

- Malignant glioma and metastatic brain cancers have a poor prognosis and effective therapies are needed.
- Many chemotherapeutics remain hindered by the BBB which has heterogeneous permeability in tumors and can remain intact in certain sites. Chemotherapeutics also can be substrates of efflux transporters or drug metabolizing enzymes.
- Strategies which circumvent the BBB may therefore improve chemotherapeutic efficacy and/or limit toxicity.
- RMT-targeted drug conjugates and nanocarriers are currently in early-Phase clinical studies for malignant glioma and metastatic brain cancers.
- A CMT-targeted nanocarrier has also reached clinical trials.
- Early-Phase clinical trials of CED-based therapies for malignant glioma are using imaging methods to monitor catheter placement and drug distribution, as well as testing novel therapeutics to enhance tumor targeting.
- Early-Phase clinical trials are underway of MRgFUS-targeted delivery of doxorubicin in patients with malignant glioma.

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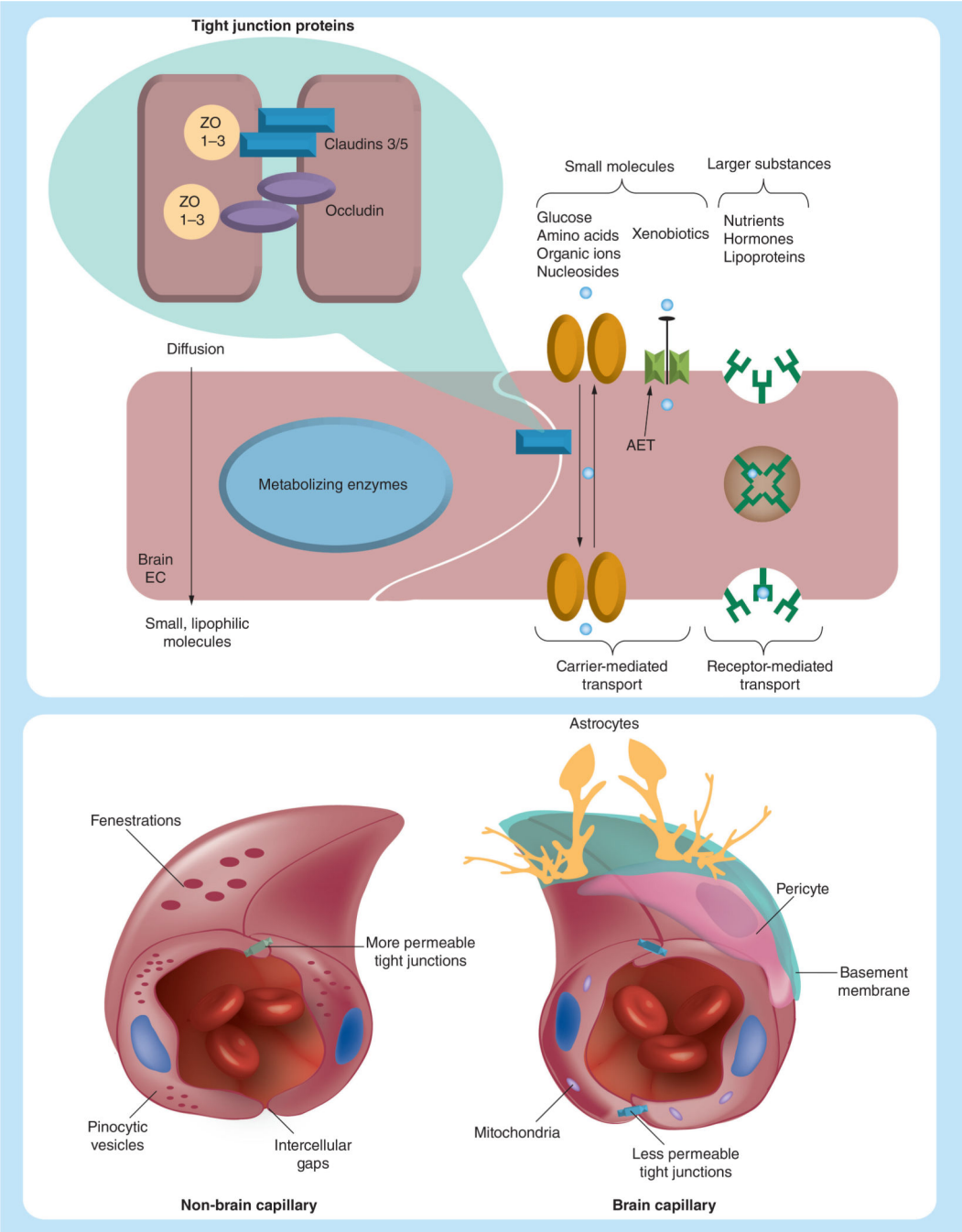

Figure 1.

See facing page. **(A)** Structure and transport across brain endothelial cells (ECs). Interendothelial tight junction complexes composed of claudins and occludin restrict paracellular diffusion, while transcellular diffusion is restricted by the EC plasma membrane. Blood-to-brain transport occurs in a selective and highly regulated manner via carrier-mediated transport and receptor-mediated transport. Additional barrier properties of brain ECs are mediated by active efflux transporters which function to transport xenobiotics in the direction of brain-to-blood and drug metabolizing enzymes. **(B)** Physiological

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differences of brain capillaries versus nonbrain capillaries. The tight junctions of brain capillaries are less permeable than in other organs. Brain capillaries do not contain fenestrations or intercellular gaps found in certain nonbrain capillaries (e.g., in kidney, liver and/or spleen). Pinocytosis is limited in brain capillaries, restricting nonspecific vesicular transport from blood to brain. Greater numbers of mitochondria are present in brain capillaries, suggesting a greater capacity for energy production to support active transport. Pericytes and astrocytes play an important role in promoting barrier function in brain capillaries.

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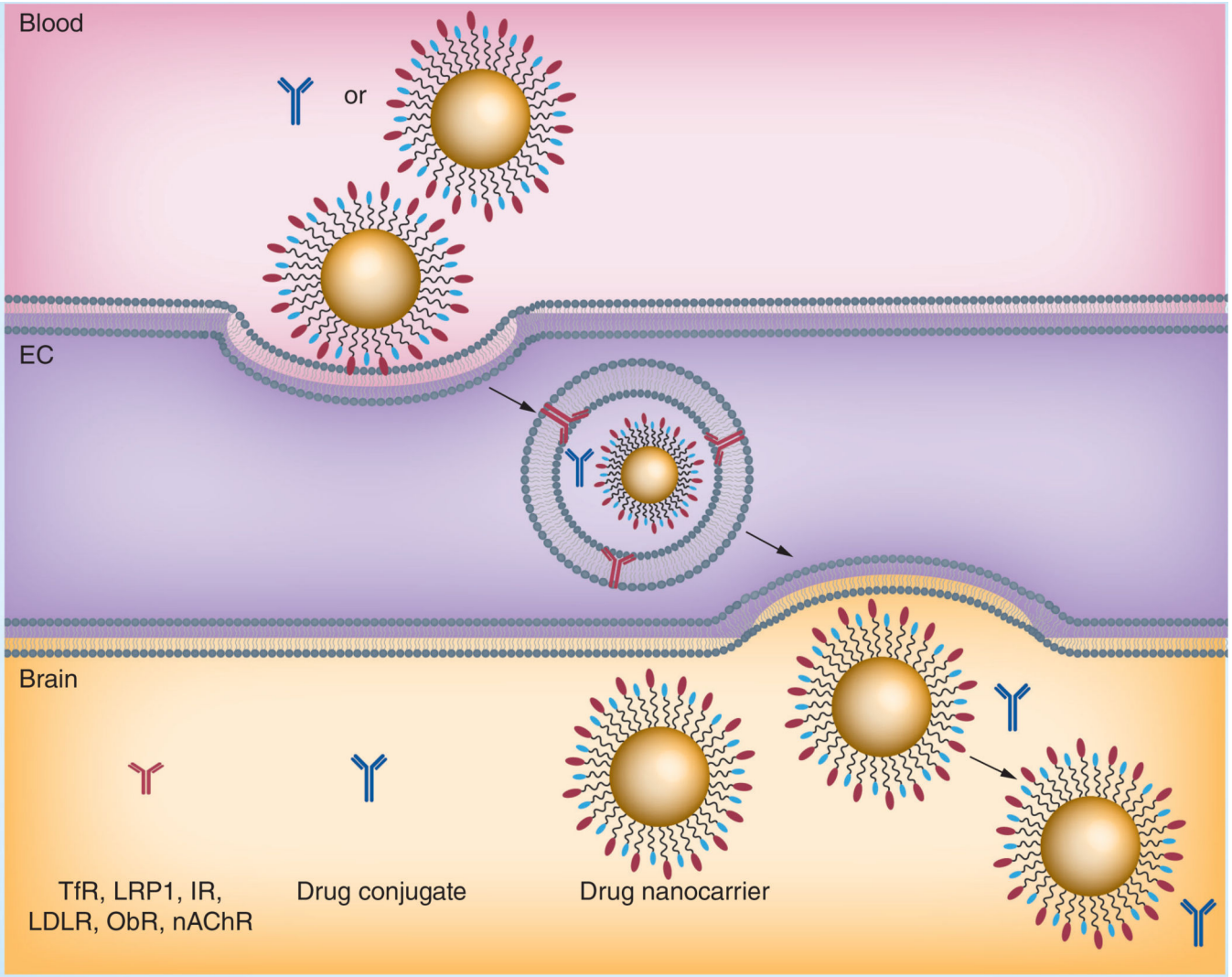

Figure 2. Receptor-mediated transport of targeted drug conjugates or drug nanocarriers across the blood–brain barrier

Targeting to endocytic cell-surface receptors present on brain ECs allows for binding and transport across the cell via vesicular transcytosis. Receptor-mediated transport receptors include TfR, LRP1, IR, LDLR, ObR and nAChR.

EC: Endothelial cell.

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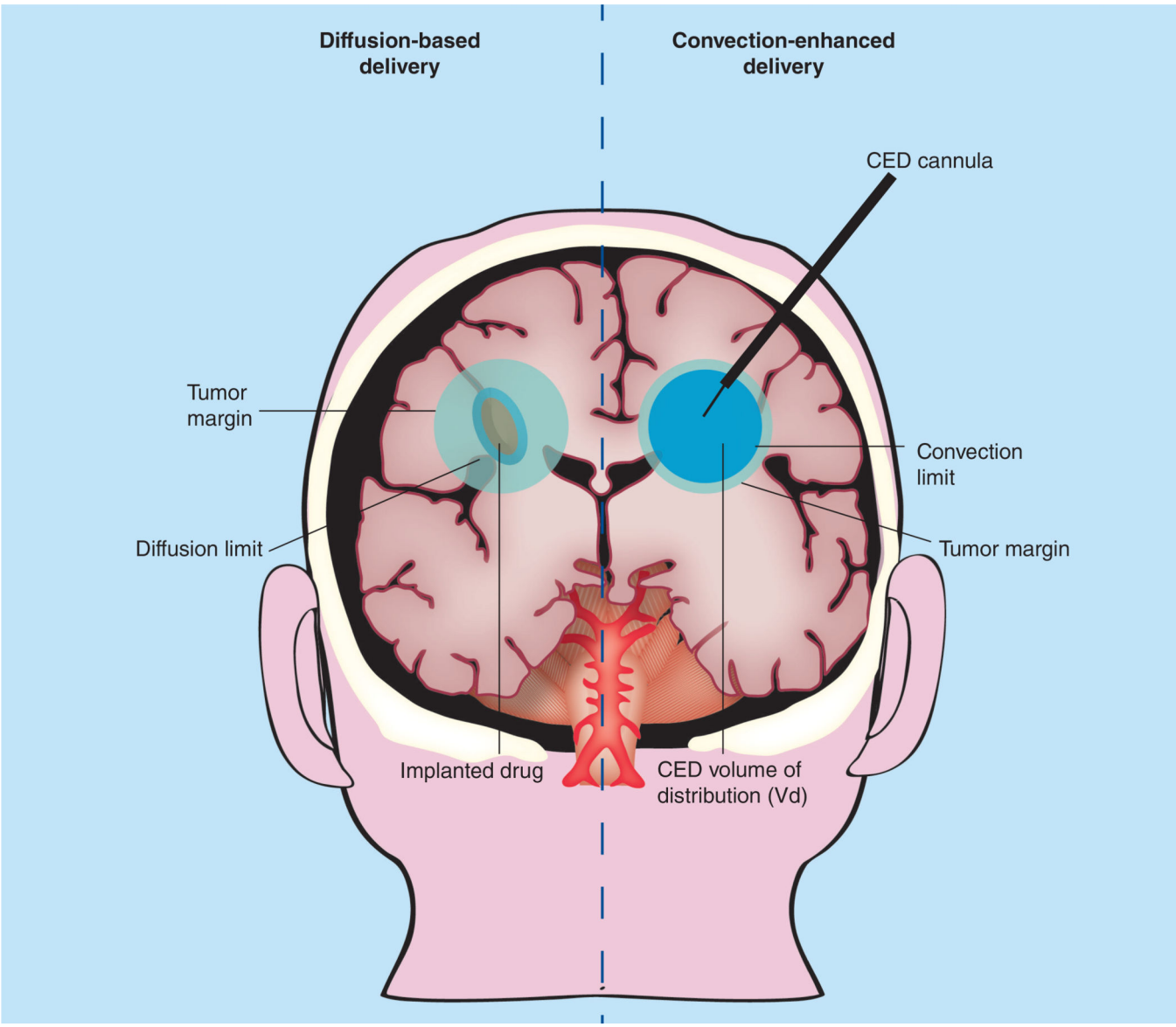

Figure 3. Comparative drug distribution via diffusion-based versus convection-enhanced delivery

The use of convective flow enables a broader distribution of chemotherapeutics.

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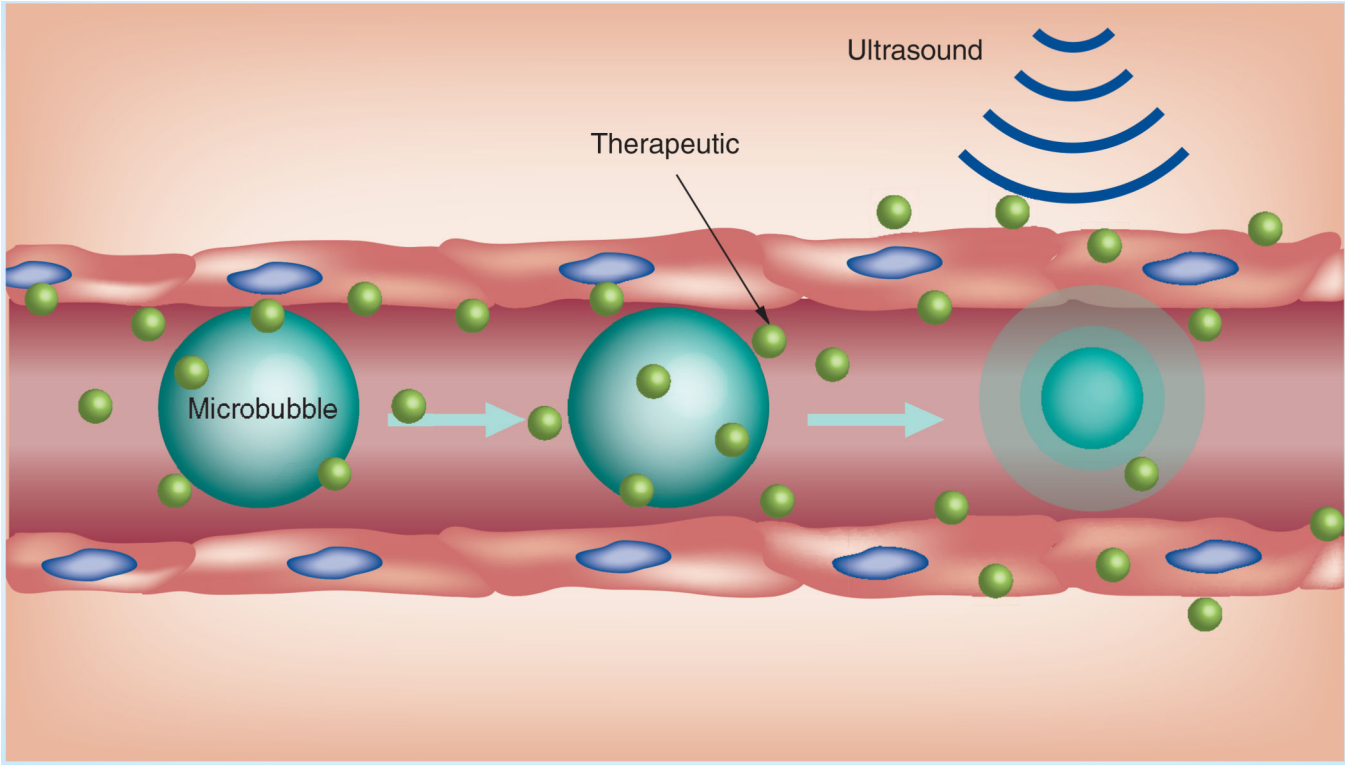

Figure 4. Blood–brain barrier disruption with ultrasound contrast agents (i.e., encapsulated microbubbles) and focused ultrasound

FUS forces microbubbles circulating in brain vasculature to oscillate nonlinearly, generating stresses that can reversibly increase the permeability of the BBB.

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Table 1

Function of blood–brain barrier tight junction molecules and cellular/molecular regulation of blood–brain I barrier permeability.

TJ molecule	Function	Intracellular binding partner	Ref.
Claudin 5	Restrict paracellular permeability	ZO-1-3	[16]
Claudin 3	Maintain BBB integrity	ZO-1-3	[17]
Occludin	Restrict paracellular permeability Regulate TJ structure	ZO-1-3	[18,33]
Signaling molecule	**Acts on**	**Effect on BBB**	
Sonic hedgehog	Sonic hedgehog receptors	• Induce expression of claudin 5, occludin • Decrease BBB permeability	[26,34]
APoE4	Occludin	Maintain TJ integrity	[31]
Ang-1	Tie2	• Decrease EC permeability • Induce PECAM-1 localization to cell junction	[30]
FGF-2	FGF receptors	• Decrease BBB permeability • Increase expression of ZO-1 and occludin	[27,28]
GDNF	GFRα1	• Increase expression of claudin 5 • Decrease BBB permeability	[29,35]
Pericyte-deficient model	N/A	• Increase BBB transcytosis • No effect on most BBB markers • Reduce polarization of astrocyte end-feet around brain capillaries	[32]

BBB: Blood–brain barrier; N/A: Not applicable: TJ: Tight junction.

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Table 2

Receptor-mediated transport-targeted peptides for brain delivery.

Peptide name (sequence)	Receptor	Derived from	Ref.
T7 (HAIYPRH)	TfR	Phage display against human TfR	[68,69]
(CRTIGPSVC)	TfR	Phage display *in vivo*	[67]
THR (THRPPMWSPVWP)	TfR	Phage display against human TfR	[69]
Angiopep2 (TFFYGGSRGKRNNFKTEEY)	LRP1	Aprotinin	[71]
ApoE3 mimic	LRP1	Human APoE3 (141–150)	[76]
G21 (TUKTIVTRINDISHTQSVSA)	ObR	Leptin (12–32)	[77]
Lep70–89(SRNVIQISNDLENLRDLLHVGGYC)	ObR	Leptin (70–89)	[78]
Leptin30 (YQQVLTSLPSQNVLQIANDLENLRDLLHLLC)	ObR	Leptin (61–90)	[77]
COG133 (LRVRLASHLRKLRKRLL)	LDLR	APoE (133–149)	[79]
Peptide-22 (cMPRLRGC)	LDLR	Phage display against human LDLR	[80]
RVG29 (YTIWMPENPRPGTPCDIFTNSRGKRASNG)	nAChR	Rabies virus glycoprotein	[81]
CDX (FKESWREARGTRIERG)	nAChR	Candoxin (snake neurotoxin)	[82]

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Table 3

Selected examples of design parameters utilized to optimize brain delivery of targeted drug conjugates or nanocarriers.

Design parameter	Drug delivery system	Effect	Ref.
Selection of target epitope	Anti-TfR antibody	Modulate selectivity for brain versus peripheral organs	[64]
Selection of target epitope	Anti-TfR antibody	Distinguish high from low grade lymphoma	[73]
Selection of target epitope	Peptide targeting LDLR	Avoid competition and inhibition of function of endogenous ligand	[80]
Nanocarrier ligand valency	Anti-TfR polymersomes	Enhance BBB penetration	[65]
Nanocarrier ligand valency	Angiopep-2 dendrimers	Enhance BBB penetration	[49]
Coupling chemistry	Anti-TfR liposomes	Enhance BBB penetration	[74]
Ligand affinity	High vesus low affinity anti-TfR antibodies	Modulate transcytosis vs intracellular/ lysosomal transport in brain ECs	[60,61]
Ligand affinity	pH-dependent anti-TfR antibody affinity	Modulate transcytosis vs intracellular/ lysosomal transport in brain ECs	[75]
Drug conjugate ligand valency	Mono- vs divalent anti-TfR Fab fragment fused to therapeutic antibody	Modulate BBB penetration	[62]

BBB: Blood–brain barrier; EC: Endothelial cell; Fab: Fragment antigen-binding: LDLR: Low-density lipoprotein receptor.

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Table 4

Clinical trials in brain cancer.

Sponsor	Treatment name/platform	Drug	Administration	BBB-targeting ligand/CMT-RMT	Phase	Disease	ClinicalTrials.gov Identifier	Ref.
Systemic CMT/RMT-based targeting								
BBB-Therapeutics B.V (Leiden, Netherlands)	2B3-101/targeted NC (PEGylated liposomes)	Doxorubicin	Systemic	GSH/GSH transporters	I/IIa	Rec/met	NCT01386580	[256]
The Netherlands Cancer Institute	2B3-101/targeted NC (PEGylated liposomes)	Doxorubicin	Systemic	GSH/GSH transporters	II	Met	NCT01818713	[257]
Synergene therapeutics (Maryland, USA)	SGT-53/targeted NC (cationic liposomes)	P53 plasmid	Systemic	Scfv/TfR	II	Rec	NCT02340156	[258]
Angiochem (Montreal, Quebec)	Ang-1005 with or without trastuzumab/drug conj	Paclitaxel	Systemic	Angiopep-2/LRP1	II	Met	NCT01480583	[251]
Angiochem (Montreal, Quebec)	Ang-1005/drug conj	Paclitaxel	Systemic	Angiopep-2/LRP1	II	Rec	NCT01967810	[252]
Angiochem (Montreal, Quebec)	Ang-1005/drug conj	Paclitaxel	Systemic	Angiopep-2/LRP1	II	Recb	NCT02048059	[253]
CED-based therapies								
University of California (CA, USA)	Liposomal irinotecan/NC (liposomes)	Irinotecan	Local	None	I	Rec	NCT02022644	[259]
Michael Vogelbaum	None	Topotecan	Local	None	0	Rec	NCT02278510	[260]
North Bristol NHS Trust	None	Carboplatin	Local	None	I	Rec	NCT01317212	[261]
Darrell Bigner	D2C7-IT/drug conj	Pseudomonas Exotoxin A	Local	None	I	Rec	NCT02303678	[262]
Darrell Bigner	PVS-RIPO/vaccine	None	Local	None	I	Rec	NCT01491893	[263]
FUS-based therapies								
Insightec (Tirat Carmel, Israel)	MRgFUS/exablate system	Doxorubicin	Systemic	None	0	Brain tumors	NCT02343991	[264]

Adv: advanced solid tumors; CMT-RMT: Carrier-mediated transporter or receptor-mediated transport receptor; Drug conj: Drug conjugate; HGG: High grade glioma; IT: Immunotoxin; Met: Metastatic brain cancer; MRgFUS: Magnetic resonance-guided focused ultrasound; NC: Nanocarrier; PVS-RIPO: Polio-rhinovirus vaccine; Rec: Recurrent high grade glioma; Recb: Recurrent brain metastasis; scFv: Single chain variable fragment.