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Is active targeting of brain metastases of breast cancer superior to passive targeting?

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Abstract

Brain metastasis is a major cause of death in patients with solid cancers. Breast cancer cells have high tendency to migrate towards brain. Cancer cells within brain are characterized by severe aggressiveness and inaccessibility. Currently, breast cancer and its metastasis are the second leading cause of death among women. Tumor microenvironment and blood brain barrier (BBB) represent great obstacles in targeting breast cancer and its metastasis. Chemotherapy is a safer treatment modality for brain metastasis compared with risky surgical resection and brain radiotherapy. Unfortunately, conventional chemotherapy lack penetration of BBB and suffer from multiple resistance mechanisms. Current treatment technologies for brain metastases of breast cancer have limited long-term success and numerous side effects, illustrating the urgent need for novel smart strategies. Various novel drug entities and nanosystems have been employed to improve diagnosis and targeted treatment of breast cancer and its metastasis. Immunotherapy agents and small tyrosine kinase inhibitors have been shown to reduce tumor size and increase survival in patients with breast cancer, but still poorly penetrate BBB. Tailored sized nanoparticles to some extent crossed brain tumor barrier and enhanced drug accumulation in tumors by taking advantage of enhanced permeability and retention. Furthermore, various active targeting strategies have been adopted to improve accessibility to brain malignancies. Therefore, to achieve enhanced antitumor therapy against

breast cancer and its brain metastasis, multi-talented delivery systems are urgently needed for optimal treatment. This review focuses on the various active and passive targeting technologies for the treatment of breast cancer brain metastases in the past decade. A comprehensive summary and examples along with pros and cons of each system will be discussed. Different treatment modalities and nanotechnology facilities will be demonstrated to aid in designing the optimal smart, safe, targeted and effective systems to combat brain metastases of breast cancer.

INTRODUCTION

Despite the recent pharmaceutical and medical advances in treatment and diagnosis of different carcinogenic tissues and their metastases, brain and central nervous system (CNS) primary and metastatic tumors remain a tough hurdle to overcome due to physiological and anatomical barriers represented by blood brain barrier (BBB) and the aggressiveness and continuous adaptive evaluation of this tumor tissue.

As systemic therapy of metastatic breast cancer advances, survival rates of patients are enhanced. On the other side, the incidence of brain and CNS complications increases[1]. In historical series, the risk of developing brain metastasis has been estimated among women with stage IV breast cancer to range from 10% to 16% among living making it the second most common cause of metastatic brain tumor after lung cancer (10%-25%)[2-4]. The development of breast cancer brain metastases (BCBM) tends to vary by the subtype. Forty-six percent of patients with advanced triple negative breast cancer, and up to thirty-seven percent of patients with HER2-positive breast cancer relapse due to intracranial metastases, despite control of the peripheral tumors[5-8].

Treatment options are limited and usually involve multimodality approaches that include surgery, radiotherapy, radiosurgery, and rarely systemic therapy, depending on the number of CNS lesions[9,10]. The unpredictable extent of therapeutics that can reach tumor vasculature of BCBM has been the challenge in developing effective treatments for these patients[11,12]. BBB has always been a challenge to drug delivery to brain tissues[13-16]. However, during brain metastases, the structure and integrity are altered forming a "Blood Brain Tumor Barrier" (BBTB)[17]. The leaky structure of BBTB is an appealing strategy to target brain tumors[18]. Approaches to deliver drugs into the brain are being extensively studied [Figure 1].

Beside the common technologies including viral vectors and nanoparticles, novel non-invasive techniques such as ultrasound alteration and magnetic stimulation have been studied to temporally open the BBB to enhance brain drug uptake. In this review, we discuss passive and active targeting BCBMs.

BBB AND DRUG DELIVERY

The BBB, as a morphologic and physiologic barrier, protects the brain tissue from the peripheral vasculature, protecting normal brain function by impeding most compounds from transiting from the blood to the brain[13,16,18]. There is a common misconception about the nature of BBB as a protective barrier or sac protecting the brain underneath it. Perhaps, the term BBB was the main reason of such misconception. The BBB is mainly a fortified special vascular structure in the brain different from the common vascular structure in the rest of the body. Mainly, the brain capillary endothelial cells (BCECs) form the BBB, besides other cell types such as pericytes, astrocytes, and neuronal cells; playing an important role in the function of the BBB[19]. BCEC have tight junctions that prevent paracellular transport of small and large (water soluble) compounds from the circulation to the brain except for some very small or gaseous molecules, such as water and carbon dioxide[17,19,20]. The components of the BBB continuously adapt in response to various physiological changes in the brain[21] [Figure 1].
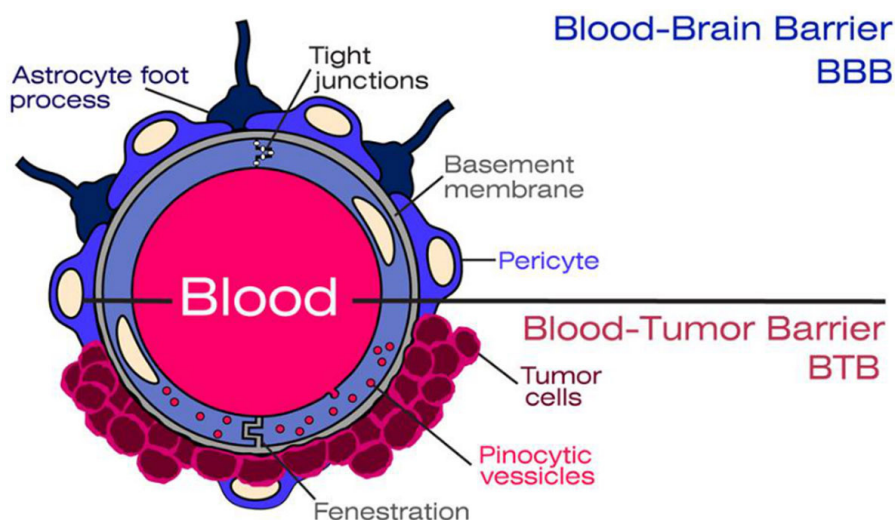
Figure 1. The vascular and structural components of the blood brain barrier (BBB) and the blood brain tumor barrier

Similarly, BCECs restrict drug distribution into the brain by limiting drug diffusion from blood to brain. In addition to these physical barriers, cells, like pericytes, and astrocyte foot processes, express several drug metabolizing enzymes that inactivate drugs reducing the distribution of active drugs to the brain. Thus, complicating barrier mechanisms & the presence of both drug-uptake and drug efflux mechanisms such as multidrug transporters including P-glycoprotein (ABCB1/MDR1) and ABCG2. Some compounds pass the BBB by passive non-saturable diffusion, some by active transport; others are actively excluded, and still, others enter through endocytosis[12].

BRAIN METASTASES OF BREAST CANCER

The occurrence of brain metastases depends on how breast cancer cells adapt to and survive in foreign environments[22]. Specific homing molecules mediate organ-specific metastasis formation on the heterogeneous tumor cell surface[23]. Investigating throughout the metastatic cascade is one area of research that can lead to the development of effective treatment.

Research has shown that at the time of initial diagnosis of primary cancer, circulating tumor cells can be found in the bloodstream of patients[24]. Breast cancer stem cells are characterized by the ability to self-renew along with their high level of resistance to radiation and chemotherapeutic agents[25]. After detachment of tumor cells from the primary lesion, invasion into the CNS and bypassing the BBB occur, followed by sustained proliferation towards the formation of brain metastases[26,27]. Studies have suggested that brain metastases originate from cells as a primary mass or lymph nodes or from other visceral metastases[28].

Disruption of the BBB was shown to involve mediators of extravasation through non-fenestrated capillaries. A number of mediators have been identified to assist breast cancer cells crossing the BBB, including cyclooxygenase-2, heparin-binding epidermal growth factor-like growth factor, and the $\alpha2, 6$-sialyltransferase ST6GALNAC5[29-34].

The recently identified extracellular (circulating or exosomal) miRNAs were shown to mediate the ability of metastatic breast cancer cells to target the distant brain endothelium and vasculature[35]. The metastatic BC cells in brain express high levels of anti-plasminogen activator serpins as a shield to escape from the reaction of brain stroma, conferring the adherence of infiltrating breast cancer cells to the surface of capillaries and the growth on the vasculature[36].
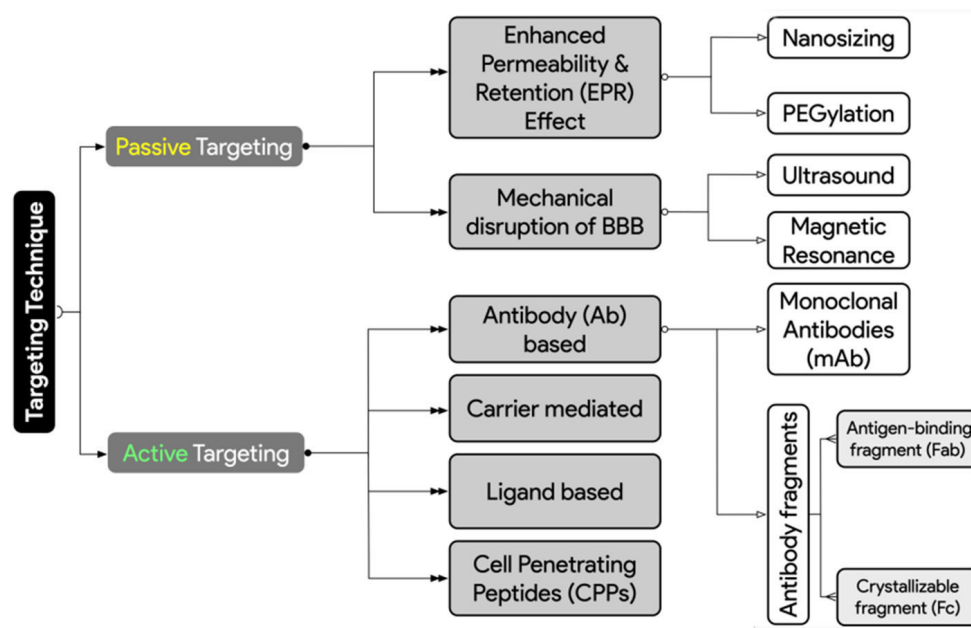
Figure 2. Different adopted strategies for actively and passively targeting brain metastases of breast cancer to overcome the blood brain barrier (BBB) hurdles

Brain metastases of breast cancer are also subject to the limited permeability characteristics of the BBB in-spite of the formation of the BBTB with altered integrity. MRI data have shown that not all brain metastases display elevated BBTB permeability[37]. The changes in BBTB vascular permeability are usually not homogenous throughout the lesion[38,39]. It was noticed that brain metastases from HER2+ breast cancers infiltrate brain tissue crossing the endothelial cells without disrupting the BBB, unlike the brain metastases from triple negative or basal-type breast cancers that often disrupt the BBB[8,40] [Figure 1].

TARGETING BRAIN METASTASES

Although, there are no FDA-approved systemic treatments for BCBM to date[41], patents and studies in the past years have shown promising progress and well-established techniques to overcome the BBB/BBTB[42]. There are two general strategies adopted to facilitate crossing the BBB; invasive and non-invasive techniques[43]. The invasive techniques rely primarily on disrupting the BBB integrity by direct intracranial drug delivery through intracerebroventricular, intracerebral or intrathecal administration, osmotic pumps or biochemical means[43]. But all these approaches are severely limited by poor distribution into brain parenchyma[44]. On the other hand, non-invasive methods include drug moieties modifications through the transformation of the drug into lipophilic analogs, prodrugs, chemical drug delivery, carrier-mediated drug delivery, receptor/vector-mediated drug delivery and intranasal drug delivery[43,45].

Diffusion of substances into the brain can be divided into paracellular and transcellular. Generally, BBB targeting strategies can be categorized into passive (transcellular lipophilic pathway) and active targeting (mainly; transcytosis)[46]. Figure 2 summarizes the targeting techniques covered in this article.

Passive targeting

Enhanced permeability and retention phenomenon
Passive targeting depends mainly on the preferential accumulation of drug molecules into tumor cells[47]. Enhanced permeability and retention (EPR) phenomenon based on the nanometer size range of the nanoparticles and two fundamental characteristics of the neoplastic tissues, namely, the leaky vasculature and impaired lymphatic drainage. EPR was first described by Maeda and Matsumura[48]. The selective high

local concentration of nanosized anticancer drugs in tumor tissues enhances the therapeutic effect with minimal side effects in both preclinical and clinical settings[49].

Various important factors such as circulation time, targeting and the capability to overcome BBB are heavily reliant on the shape, size and the surface area of these particles. In passive diffusion, lipid solubility, molecular weight, and the presence of tight junctions greatly affect the degree of permeation[50,51].

Conventionally, a particle must be at least 10 nm in diameter to avoid clearance by first-pass renal filtration and the optimal size range of 100-180 nm will ensure longer circulation time, increased accumulation within the tumor mass and lower renal clearance[52,53]. Moreover, surface characteristics also play a very important role in determining the extent of internalization of these nanoparticles into cells. Relatively, the surface can be modified by the polymer composition, thus governing an extra amount of hydrophobicity or hydrophilicity to these particles[51]. Supplementary Table 1 illustrates some of the papers using passive targeting technology in targeting BCBM.

Nanotechnology as a tool for targeting EPR
Nanotechnology paved the road towards safer and more likely effective cancer treatment strategies that can overcome some of the hurdles for drug delivery. Nano-medicines primarily aim to improve the circulation time of the conjugated or entrapped (chemo-) therapeutic drugs[54]. Examples of nanoparticles commonly used include: polymeric particles, polymeric micelles, dendrimers, and liposomes. Nanoparticles represent versatile tools to encapsulate various types of drugs, either hydrophilic or hydrophobic moieties altering their physicochemical parameters and pharmacokinetics profile[55].

Beside increasing drug-site contact time, some polymers used in nanoparticles formulations such as polylactic-co-glycolic acid (PLGA) have high cell adhesion property, increasing the drug concentration gradient at the adhesion site by longer drug carrier contact time with the targeted cells[56].

However, nanoparticles can show some serious adverse effects[57]. Adverse effects of nanoparticles depend on individual factors such as genetics, existing disease conditions, exposure, nanoparticle chemistry, size, shape, agglomeration state, and electromagnetic properties[57]. The key to understanding the toxicity of nanoparticles is their size; nanoparticles are smaller than cells and cellular organelles, which allow them to penetrate these biological structures, disrupting their normal function[57]. Examples of toxic effects include tissue inflammation, and altered cellular redox balance toward oxidation, causing abnormal function or cell death[57]. Furthermore, a major drawback is the difficulty in scaling up the formulation and its transformation to clinical use, due to high cost and instability during storage. The NP materials must be biocompatible and safe when administered[58].

Lipsomes
Liposomal (Liposome: lipid vesicle in Latin) nanocarrier systems are vesicular lipid bilayer colloidal spheres formed by self-assembly[59]. Without the use of surface modification by a hydrophilic polymer such as polyethylene glycol (PEG), the biological half-lives of liposomes are very short due to several factors including the tendency of the liposome to exchange lipid materials with cell membranes and their uptake by phagocytes. Mohammad *et al*.[60] noticed increased survival rates in animals treated with liposomal irinotecan (nal-IRI) that sustained the accumulation of irinotecan and its active metabolite in the brain metastases lesions, unlike non-liposomal irinotecan whose distribution in the CNS was hampered by BBTB and various efflux proteins. The size of nal-IRI was between 100-110 nm, which enhanced its preferential accumulation within tumor due to EPR through the leaky vasculature of the tumor[60]. Drawbacks of liposomes involved their rapid uptake by the Reticulo-Endothelial System (RES) and consequent removal from circulating blood.

PEGylation

The main pharmacokinetic outcomes of PEGylation include: changes occurring in overall circulation life-span, tissue distribution pattern, and elimination pathway of the parent drug/particle[61]. PEG mainly protects these particles from being phagocytosed by natural particle eliminating mechanisms, mainly organs of the RES[62].

Pegylated nanoparticles are characterized by hydrophilic surfaces that circumvent opsonization and decrease their clearance by macrophage, which results in prolonged circulation[51]. PEGylation has been shown to alter the pharmacokinetics of doxorubicin considerably; the total clearance was significantly reduced[63]. In the early 1990's, PEGylated polymeric vesicles were introduced by Yokoyama *et al*.[64], which represented an important milestone in the synthesis of long-circulating liposomal formulations (STEALTH® liposomes). Liposomal formulations are the first novel controllable carrier systems to be sold in the market for cancer (Doxil©, PEGylated liposomal formulation encapsulating doxorubicin(PLD)[65-67]. PLD showed reduced cardiotoxicity and prolonged activity.

Anders *et al*.[68] encapsulated doxorubicin in pegylated liposomes (PLD) that achieved 20 folds higher concentration of doxorubicin within intracranial tumors compared to non-liposomal doxorubicin, whose distribution was compromised by BBTB. Moreover, co-administration of PLD and BBB permeable ABT-888, an inhibitor of a poly (ADP-ribose) polymerase, showed better survival as compared to non-liposomal doxorubicin and ABT-888.

Nektar™ Therapeutics has several patents on PEGylation bioconjugation aiming to modify the pharmacokinetic profile[69-75]. Adkins *et al*.[76] formulated NKTR-102, an Irinotecan-PEG conjugate linked with a hydrolysable ester bond, whose polymer moiety resulted in prolonged circulation and subsequently increased tumor localization. The innovative nature of this PEGylated system is the adoption of the three-dimensional (3D) branching technology in the PEGylation to provide superior pharmacokinetics and pharmacokinetic parameters. The 3D propriety Nektar PEG technology offers weeks of half-lives compared to days in conventional PEG[76]. The preferential accumulation of NKTR-102 within brain metastases via EPR, its reduced clearance and ability to escape P-glycoprotein mediated efflux resulted in continuous release of the active metabolite SN38 and better therapeutic efficacy.

Nektar™ Therapeutics is not the only pharmaceutical company relying on innovative PEGylation technologies for passively targeting brain metastases of breast cancer. Other companies, such as 2-BBB, is investing heavily on the use of PEGylation bioconjugated with liposomal vesicular carrier systems to passively target brain metastases of breast cancer[77]. BBB adopts a propriety G-Technology® for targeting the brain. 2-BBB's G-Technology® empowers sustained delivery of systemically administered therapeutics to the brain with high safety and efficacy profiles. The G-Technology® uses PEG and glutathione bioconjugated to active pharmaceutical ingredients loaded liposomal vesicular structures. Polyethylene glycol (PEG) is attached to the liposomes to provide a prolonged circulation time in the blood stream with sustained half-life pharmacokinetic profile. Glutathione is bioconjugated to the PEG molecules to offer targeted safe and effective delivery of the therapeutics across the BBB[78].

2-BBB has adopted its G-Technology® in developing two lead clinical programs targeting multiple indications of brain cancers and neuroinflammatory diseases. 2-BBB's lead product 2B3-101 (glutathione PEGylated liposomal doxorubicin) combines the G-Technology® with the existing chemotherapeutic agent doxorubicin for the possible treatment of brain metastases and glioma. It has completed a Phase I/IIa trial treating patients with various forms of brain cancer. 2-BBB's second promising lead product is 2B3-102. 2B3-102 is a glutathione PEGylated liposomal methylprednisolone. It applies the anti-inflammatory glucocorticoid methylprednisolone to combat acute and chronic neuro-inflammation associated with several CNS

indications including acute relapses of multiple sclerosis, optic neuritis, neuromyelitis optica and uveitis. 2B3-201 has completed a Phase I clinical trial in healthy volunteers[77].

2-BBB is a clear example of industry-driven research aiming at developing innovative passive targeting technologies to brain and brain metastases. They use triple passive targeting techniques to offer optimal safety and efficacy profiles, Glutathione passively targeting the cancerous intracellular reductive environment, PEGylation and liposomal vesicular carrier system[77].

Nucleic acid

RNA interference (RNAi) is an endogenous pathway for post-transcriptional silencing of gene expression that is triggered by double-stranded RNA, including endogenous microRNA (miRNA) and synthetic short interfering RNA[79]. MicroRNA-122 mediated RNAi brings new prospects[80]. Zhang *et al*.[81] miRNA-1258 in BMBC cells inhibited heparanase which regulates many molecules involved in angiogenesis and metastasis of the tumor.

Advanced physically manipulated systems to disrupt BBB

Another modality of addressing BBB passively is advanced physically manipulated systems that can be tightly mediated by stimuli to treat diseases specifically and with a controlled dosage of drugs. Physical manipulation can be achieved based on ultrasound, electricity, magnetism and photonic emission technologies[82]. Davalos *et al*.[83] applied pulsed electric fields into brain tissue of an animal, to cause temporary disruption of the BBB in a volume of brain tissue near the source of the pulsed electric fields over a specified time interval.

The use of focused ultrasound (FUS) combined with circulating microbubbles is a non-invasive method that increases the permeability of BBTB and improves outcomes of trastuzumab; this technique was used by Park *et al*.[84] to improve outcomes with trastuzumab in a breast cancer brain metastasis model. Similarly, FUS in combination with microbubbles was able to temporarily disrupt the BBB enhancing the anti-tumor efficacy of the two anti-HER2 agents combination therapy[85]. Moreover, reversible disruption of the BBB by bursts of low frequency MRI-guided ultrasound enhances the brain delivery of monoclonal antibody - Herceptin (trastuzumab) in mice[86,87].

Active targeting

Active targeting was proposed for improved targeting efficacy[47]. These strategies consist in incorporating affinity molecules or taking advantage of influx transport systems expressed within the BBB/BBTB depending on specific interactions of ligand-receptor and antibody-antigen[47,88,89]. Also, several ligands have been studied and utilized to shuttle nanoparticles, antibodies, and drugs across the BBB and into the brain cells[90] [Figure 1]. Supplementary Table 2 summarizes recent studies of actively targeted treatments for BCBM.

Receptor-mediated transcytosis/ligand-based

An overexpression of receptors or antigens in cancer acts as a potential target to achieve efficient drug uptake via receptor-mediated endocytosis[47]. Moreover, receptor-mediated transcytosis (RMT) allows for BBB transport of various macromolecules after initial binding of a targeting ligand to a receptor expressed on the brain endothelial cells[91]. Tumor-targeting ligands such as peptides and antibodies may effectively aid certain cytotoxic agents (either biological or synthetic) to deliver to the tumor cells, thereby improving therapeutic efficacy while limiting the exposure of normal tissues to the cytotoxic agents[92]. The transferrin receptor, the low-density lipoprotein receptor-related protein 1 (LRP-1), the insulin receptor and the nicotinic acetylcholine receptors are examples of receptor expression on the BBB.

AngioChem[TM] Inc. developed a series of aprotinin polypeptides (Angiopeps)[93]. Angiopep-2, a 19-amino-acid peptide, is one of the promising vectors designed to target the LRP-1 receptor, to mediate transcytosis across the BBB. It is derived from the human Kunitz domain[94]. Angiopep-2 can facilitate brain-targeted drug delivery through LRP-1-mediated transcytosis. Regina *et al*.[95] demonstrated that a conjugate between angiopep-2 and an anti-HER2 mAb results in a new chemical entity, ANG4043. ANG4043 retains *in-vitro* binding affinity for the HER2 receptor and antiproliferative potency against BCBM rat model. This study showed increased uptake in brain endothelial cells and enhanced BBB permeability compared to poor brain penetration of anti-HER2 mAb alone. Similarly, Thomas *et al*.[96] showed that ANG1005 shows significantly improved delivery to brain and brain metastases of breast cancer compared to free paclitaxel in mice bearing BCBM.

On the other hand, Orthmann *et al*.[97] formulated rigid and fluid liposomes entrapping Mitoxantrone and equipped with a 19-mer angiopeptide as a ligand. Angiopeptide bearing fluid liposomes showed *in vitro* the highest cellular uptake and transcytosis. They were significantly better than the corresponding ligand-free fluid liposomes and ligand-bearing rigid vesicles however; the improvement was mainly depending on liposomal fluidity while the targeting contributed only to a minor degree. In 2016, Orthmann *et al*.[98] encapsulated oxaliplatin (OxP) in liposomes then bound angiopep-2 to the vesicular surface. They determined that the newly developed OxP liposomes significantly improved the treatment of subcutaneously and intracerebrally growing breast cancer, but the targeted angiopep-equipped liposomes showed no superior effect *in vivo*.

Melanotransferrin (hMTf) is another target to the LRP-1 receptor, which was shown to deliver doxorubicin across BBB[99,100]. In order to overcome the trastuzumab inability to cross the BBB and treat brain metastases of HER2+ breast cancer, BT2111, a novel bioconjugate of trastuzumab was developed by BiOasis Inc., on the hMTf (p97) Transcend[TM] vector platform[101]. This platform is an example of an actively targeted immunotherapy, aiding in enhancing the immunotherapy targetability, ability to cross the BB and clinical efficacy via the use of the targeting moiety hMTf.

Another complex process involves binding to a primary, tumor-specific receptor activating endocytosis; using "Tumor-penetrating peptide"[102]. The prototypic peptide of this class, iRGD (CRGDKGPDC), contains the integrin-binding RGD motif[103]. The integrin-binding RGD sequence motif binds to $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins, which are specifically expressed in tumor endothelial cells[102]. Hamilton *et al*.[104] demonstrated that a single dose of iRGD had a significant effect on metastatic tumor progression and nonproliferative cancer cell retention when applied early in course of tumor development. Proteolytically processed iRGD also exerts anti-metastatic activity by binding to neuropilin-1 and activating an endocytic bulk transport pathway through tumor tissue. The iRGB platform offers an innovative dual targeting tool to target the tumor portion via the integrins and the RGB motif[103].

Carrier-mediated transcytosis
Naturally, carrier-mediated transcytosis (CMT) enables spontaneous internalization of small biomolecules[105,106]. CMT takes advantage of the immunological surveillance system of the brain, using circulating phagocytic cells such as monocytes or macrophages as Trojan horse to deliver drug molecules into the brain[107]. Such cells have a tendency to endocytose colloidal materials, for example, nano or microparticles, liposomes, and subsequent exocytosis to release drug and/or colloidal materials to external media[108]. Fidler and colleagues provided evidence that macrophages of blood monocyte origin can infiltrate experimental brain metastases while the BBB is intact.

In 2012, Choi *et al*.[109] reported the first successful demonstration of the active delivery, using macrophages, of nanoparticles to brain metastases. Activated macrophages not only cross the BBB but they envelop the

metastatic cells delivering the loaded nanoparticle to less than a cell width away from the nearest metastatic cell[109].

Cell penetrating peptides

Cell-penetrating peptides (CPP), that show great capacity in BBB transport, have the ability to transport protein or peptides into cells in a nonspecific, receptor-independent manner and non-immunogenic when compared with antibodies[110]. Considering their smaller size (up to 30 amino acids in length), cationic and/or amphipathic CPPs have a greater potential to penetrate the BBB than other transport systems. Short peptides, as targeted drug delivery vehicles, appear to have some advantages owing to their small size, efficient tissue penetrability, and minimal toxicity and immunogenicity.

The first CPP, trans-activator of transcription (TAT), derived from human immunodeficiency virus-1, can be efficiently taken up from the surrounding media[111]. Morshed *et al*.[112] used PEGylated gold nanoparticle conjugated to TAT peptide as well as doxorubicin. This formulation offered extensive accumulation of particles throughout diffuse intracranial metastatic microsatellites. Moreover, it was shown to destabilize a brain capillary monolayer increasing its permeability. Fu *et al*.[113] combined brain metastatic breast carcinoma cell (231-BR)-binding peptide BRBP1, a cell penetrating peptide TAT, and a proapoptotic peptide KLA. The composite selectively homed to the tumors in vivo where it induced cellular apoptosis without significant toxicity on non-tumor tissues. Angiopep-mediated targeting also can be considered as one of the most promising ways to reach the CNS for the treatment of brain cancer or brain metastases[114].

Conclusion and future directions

There is a global need for effective and safe pharmaceutical chemotherapeutic agents that have the potential to target tumors like BCBM.

Conventional chemotherapy, radiation therapy and immunotherapy offers promising options for treating brain metastases, which is traditionally treated with surgery.

In 2005, the chemotherapy temozolomide (Temodar®) was approved to treat glioblastoma (GBM) patients. However, over 50% of GBM tumors generate a DNA repair protein (methylguanine methyltransferase) that effectively combats and neutralizes temozolomide chemotherapy. There are two FDA approved immunotherapies for brain and nervous system cancers and metastases. Bevacizumab (Avastin®) and Dinutuximab (Unituxin®). Several other immunotherapies are being used to treat different types of brain cancers in clinical trials. However, there are no FDA-approved systemic treatments specific to BCBM to date[41]. Improved insights into the microenvironment and metastatic cascade processes have resulted in the development of several novel chemotherapeutic and immunotherapeutic drugs and strategies[35,36,38,39,54]. Such novel chemotherapeutic and immunotherapeutic strategies adopt active and passive targeting strategies to enhance the clinical treatment effects.

Surveying the published research papers and patents also revealed a major drawback in the methodology design, which is the lack of standardization of efficacy and safety profiles. Various sophisticated nano-systems and conjugates have been studied to either actively or passively target BCBM. However, most of these complex delivery systems fail to reach the market due to high cost, instability and difficulty of scaling up. In addition, majority of the reagents used in the formulation of such novel therapeutic systems to improve the stability; are not included in the FDA approved inactive ingredient database. The main question of this review is to query the preferential advantage of either active or passive targeting in combating brain metastases of breast cancer. Such question is a major ongoing debate as revealed by the recent patents and papers portfolio.

Passive targeting could be a cheaper, long-term effective and safe technique to target tumor cells without triggering resistance for active targeting moieties. Numerous significant downsides in active tumor-targeted drug delivery were identified[115-120]. Interestingly, passive targeting can be tailored and designed via simple ideologies and techniques such as simple PEGylation, size modifications or even shape customization. It was shown that slender shape and particles modifications such as drifting from the conventional spherical particulate structures, can yield high effectual passive targeting[121].

Active targeting shows significant results in combating cancer initially with long-term degraded efficacy profile, most potentially attributed to multidrug resistance[119]. Additionally, the marginally augmented efficacy of active targeting strategies does not substantiate the extremely higher cost, difficulty in clinical application and the high complexity level of the carrier system[54,78,115-120,122,123]. Interestingly, passive targeting can be tailored and designed via simple ideologies and techniques such as simple PEGylation, size modifications or even shape customization.

Multiple researchers suggested the preferential long-term advantage of passive targeting over active targeting such as Lammers *et al*.[122] and Rosenblum *et al*.[123]. Such researches highlighted the need for future realistic and scalable efforts to address some of the conceptual drawbacks of drug targeting to tumors such as resistance, and that strategies should be developed to overcome these deficiencies. Such research presented evidence that passive targeting yield very comparable results to active targeting on the short term and superior results on the long term[54,78,115-120,122,123].

Some of the main drawbacks of engineered nanotechnologies, either active or passive, are the poor robust and cost-effective toolbox to characterize nanomaterials and to quantify exposure in test systems (dosimetry), lack of standard techniques and methods for test, hazard, and risk assessment strategies and lack of specific safety assessment strategies in evaluating nanotoxicities. However, passive targeting offers less problematic strategy compared to active targeting engineered nanotechnologies in terms of nano-safety and its quantitative characterization[124].

Passive targeting could provide an applied direction for the advance of novel management tackles and therapeutics for brain metastases of breast cancer for researchers worldwide, paving the road to affordable, scalable, stable, efficient and safe management strategies. Such observations were evident from the multiple passive targeting products under Phase II and Phase III clinical trials from multiple pharmaceutical firms such as Nektar Therapeutics, BiOasis Inc. and 2-BBB. Such companies have invested heavily into the development of new formulations that combat brain barriers, targeting the brain and accumulates in tumor tissue rather than normal cells, to enhance the nano-safety profiles to chemotherapeutics. This may not be only attributed to their need to expand their portfolio to increase their commercial value for possible products buyouts and licensing by big pharmaceutical firms, but the safety and efficacy potential of such passively targeted nanotherapeutics in the treatment strategies of brain metastases of breast cancer compared to current conventional modalities and research-driven active-targeting initiatives.

Such conclusions have driven the industry to invest heavily on passive targeting compared to active targeting. A major focus by industry is the successful transformation of such novel technologies from bench to bedside with reasonable cost, scale-up abilities and formulation robustness and reproducibility, which are achievable via passive targeting technologies compared to active targeting. Active targeting ligand post-insertion and labeling techniques need to be extensively researched for ease of application for active targeting to gain industrial momentum. Conclusively, patents and products under development should focus on simple passively targeted bioconjugate structures, which are easily synthesized with high yield, reduced cost and high stability profile of the final formulation.

DECLARATIONS

Authors' contributions
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