Nanomedicine

Nanomedicines in the treatment of brain tumours

Journal:	*Nanomedicine*
Manuscript ID	NNM-2017-0378
Manuscript Type:	Editorial
Keywords:	Brain cancer, Nanomedicine, Theranostic

Nanomedicines in the treatment of brain tumours

Funmilola A. Fisusi[1,2], Andreas G. Schätzlein[1,3], Ijeoma F. Uchegbu[1,3*]

[1]UCL School of Pharmacy, 29 – 39 Brunswick Square, London WC1N 1AX

[2]Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

[3]Nanomerics Ltd., New Bridge Street House, 30-34 New Bridge Street, London EC4V 6BJ

* Author for correspondence

Keywords

Brain tumour, nanomedicine, nanoparticle, theranostic, glioblastoma, doxorubicin, paclitaxel

Brain metastases and primary CNS tumours

Brain metastases are the most frequently occurring neurologic complications of cancer in adults, with 9 – 17% of all cancers resulting in brain metastasis and brain metastasis occurring in 8 – 14 per 100,000 in the general population [1]. Primary brain tumours, on the other hand, are relatively rare, and comprise about 1.4% of cancers [2]. Brain metastases are associated with a median survival times of about 3 – 25 months [3], and a 5 year survival rate of 1.8% [4]. Treatment modalities employed for brain metastases include: surgical resection, whole brain radiation therapy, radiosurgery and chemotherapy [5]. The choice of treatment would usually be based on several considerations. These include: histopathology of the primary tumour, status of systemic disease, patient's performance status (general well being and lifestyle activity level), age of the patient, number and sites and precise location of the

brain metastases (such as proximity to sites of vital brain function), co-existing morbidities, and symptoms [2,5].

Glioblastoma multiforme (WHO Classification astrocytoma Grade IV), a metastatic primary brain tumour, accounts for 12 – 15% of all brain tumours [6] and is the most common primary brain tumour in adults [7]. Glioblastoma is an aggressive metastatic astrocytoma with a median survival of 14 months and less than 5% of patients survive for 3 years [8]. This tumour is difficult to diagnose early as the tumour is usually asymptomatic or presents with symptoms which are difficult to associate with GBM, e.g. symptoms associated with a high intracranial pressure (headaches, nausea, vomiting and cognitive impairment) [9]. A major contribution to the poor survival rates is the insufficient transport of therapeutic molecules across the blood brain barrier (BBB) [10]. The current standard of care comprises surgical resection to the maximum possible extent, followed by concurrent radio–chemotherapy and adjuvant chemotherapy with temozolomide [2]. This treatment regimen became the standard of care for newly diagnosed glioblastoma patients after the results of the 2004 European Organisation for Research and Treatment of Cancer 26981-22981/ National Cancer Institute of Canada Clinical Trials Group CE3 randomised phase III trial demonstrated a 20.7% improvement in the median survival as well as 27.2% two-year survival rates in glioblastoma patients, who had received post-surgical concomitant and adjuvant temozolomide (known as the Stupp regimen) compared to 10.9% two-year survival rates with post-surgical radiotherapy alone [11]. For recurrent glioblastoma on the other hand, there is currently no standard treatment regimen [12], and thus patients frequently receive investigational agents in clinical trials [13].

The Blood Brain Barrier (BBB)

The treatment of brain tumours (or more generally, central nervous system (CNS) tumours) is particularly challenging, mainly because of their intracranial location [14]. Intracranial

tumours are effectively "shielded" from the effects of most systemically administered cytotoxic agents. The brain parenchyma and most (but not all) intracranial tumours are protected by the intact blood brain barrier (BBB), which maintains the brain microenvironment by serving as a physical and metabolic barrier regulating the access of molecules to the brain [15]. The physical barrier is formed by the tight junctions between the adjacent endothelial cells (which prevent blood-borne substances from crossing into the brain parenchyma), a lack of capillary fenestrations, very low pinocytotic activity and the metabolic barrier is formed by degradative enzymes, specialised transport receptors and endothelial cell efflux pumps [15].

Other Brain Tumour Treatment Barriers

Another barrier thought to restrict access of systemically administered therapeutic agents to tumour cells is the brain tumour-cell barrier (BTB, a barrier due to the efflux activity of tumour cells) [16]. Other challenges associated with effective brain tumour treatment are: dose limiting toxicity, mainly myelosuppression and tumour resistance to alkylating agents; the latter mediated mainly by the overexpression of O^6-methylguanine-DNA-methyltransferase (MGMT), a ubiquitous protein encoded by the MGMT gene [14].

Passive Targeting with Nanoparticles

Nanoparticles have been used to passively target drugs to intracranial tumours, on intravenous injection, in order to enable delivery of therapeutics across the BBB to the brain, as there is evidence that nanoparticles are able to preferentially accumulate drug at tumour sites, when compared to the administration of drugs in solution [17]. Generally nanoparticles may be engineered to: a) enable tissue or organ specific transport of their drug payload, or b) enable the delivery of hydrophobic and metabolically labile drugs [18,19]. Thus, nanoparticles are an interesting platform to consider in drug development for brain tumour indications [19].

Intravenously administered nanoparticles for delivery of therapeutic agents to brain tumours may theoretically exploit the enhanced permeability and retention (EPR) effect, whereby particles extravasate through a leaky tumour vasculature and achieve closer proximity to the tumour cells [20]. However for the EPR effect to be operational, the BBB must be compromised at the site of the intracranial tumour and while the breakdown of the BBB is diagnostic of a high grade glioma [21] most tumours are associated with an intact BBB [22] and direct evidence of nanoparticle accumulation within intracranial tumour cells is difficult to find. Early activity in this area focused on the delivery of P-gp efflux pump substrates to the brain in an attempt to circumvent the blood tumour-cell barrier. The P-gp substrate [23], doxorubicin, when intravenously injected in poly(butylcyanoacrylate) nanoparticles, resulted in increased tumour tissue accumulation, in a mouse C6 glioma rat model, when compared to healthy tissue and an attendant improvement in tumouricidal activity was observed with these nanoparticles when compared to the drug in solution [24]. Additionally, the formulation was also found to be less cardiotoxic. This provides indirect evidence that nanoparticles are able to take advantage of a variation in the BBB at the tumour site.

As well as the cyanoacrylates, other polymers have also demonstrated the tumour tissue drug accumulation phenomenon on intravenous injection. Doxorubicin loaded on to poloxamer 188-coated poly-(lactic acid-co-glycolic acid) (PLGA) nanoparticles in a rat glioblastoma 101/8 model resulted in superior tumouricidal activity, through the intravenous route, when compared to the drug in solution [25].

Nanomedicines may also consist of more than one therapeutic for the treatment of brain tumours. For example, chitosan surface modified PLGA nanoparticles loaded with carmustine along with O^6-benzylguanine (which depletes MGMT, thus improving the therapeutic efficacy of carmustine). On intravenous injection, this nanoparticle formulation yielded superior survival outcomes in F98 glioma-bearing rats compared to the administration of the two drugs separately in solution or to the nanoparticle containing carmustine alone [26].

Nanoparticles may also work by simply increasing plasma exposure, which in turn increases brain exposure, while minimising exposure to areas of potential toxicity [27]. We have shown that lomustine loaded on to GCPQ (N-palmitoyl-N-monomethyl-N,N-dimethyl-N,N,N-trimethyl-6-O-glycolchitosan) nanoparticles resulted in increased plasma and brain exposure, reduced liver and bone exposure and ultimately increased tumouricidal activity

(survival and tumour size) in a U87MG intracranial tumour model, without increasing myelosuppression [27].

Active Targeting with Nanoparticles

Active targeting involves the use of carriers bearing various surface ligands to achieve either transport across an intact BBB, or alternatively, cell uptake following extravasation across a leaky BBB [28].

Various across BBB transporters have been exploited for transport across an intact BBB, such as the transferrin receptor [29] and the GLUT receptor [30]. Transferrin – cyclo-[Arg-Gly-Asp-d-Phe-Lys] - c[RGDfK] paclitaxel micelles have been prepared and injected intravenously to a U87MG mouse model, with transferrin included to enable across BBB transport while c[RGDfK] was included to enable uptake by tumour cells [29]. This resulted in drug accumulation in the brain and a superior anti-glioma effect compared to the commercial formulation, Taxol [29]. Others have utilised the T7 peptide (HAIYPRH) to target the endothelial cell transferrin receptor and achieve across BBB transport [31]. T7 peptide modified core-shell nanoparticles (T7-LPC/siRNA) have been shown, on intravenous administration, to accumulate anti-epidermal growth factor receptor (anti-EGFR) siRNA in intracranial tumour tissue, down regulate EGFR and increase survival rates in a U87MG mouse tumour model, when compared to plain nanoparticles [31]. While the T7 peptide appears to achieve delivery across the BBB [31], efforts to improve cell uptake, once extravasation has taken place, have involved the use of dual targeting strategies, in which transport across the BBB is combined with a ligand promoting tumour cell uptake [32]. Intravenously administered dual targeted doxorubicin liposomes comprising the TAT peptide (AYGRKKRRQRRR) for cellular uptake and the T7 peptide for across BBB transport resulted in increased delivery of doxorubicin to the brain glioma tissue in a C6 glioma mouse model and reduced delivery to the heart, which is relevant for the cardiotoxic [33] drug doxorubicin [32].

Utilisation of the GLUT receptor to cross the BBB has been achieved by using 2-deoxy-D-glucose modified poly(ethylene glycol)-co-poly(trimethylene carbonate) paclitaxel nanoparticles [30]. The 2-deoxy-D-glucose moiety was correlated with drug accumulation in the brain and these glucose-decorated nanoparticles produced superior survival in an RG2 mouse glioma model, when compared to plain nanoparticles and Taxol [30].

While there is good preclinical evidence showing the efficacy of nanoparticles in rodent models of intracranial tumours, clinical evidence on the use of nanoparticles is harder to locate. There are some reports of clinical trials in brain tumour patients with passively targeted nanoparticles (Table 1): e.g. NCT02340156, NCT02820454, NCT01266096, NCT03020017, NCT00734682 [34,35], however the efficacy of this nanoparticle approach in the clinic has not yet been reported.

Table 1: Clinical studies on intravenously injected nanoparticles in brain cancer

Study number	***Nanoparticle type***	***Drug***	***Indication***	***Study Phase***	***References***
NCT02340156	Cationic liposomes	Liposomes encapsulated p53 cDNA in combination with oral temozolomide	Recurrent Glioblastoma	Phase II	Ref. 35
NCT02820454	Polymer-gadolinium chelates	AGuIX (polysiloxane gadolinium-chelates based nanoparticles) concurrently with whole brain radiation.	Brain metastases	Phase I	Ref. 35
NCT01266096	Silica	124I-cRGDY-PEG-dots for positron emission tomography	Newly diagnosed or recurrent metastatic melanoma, malignant brain	Microdosing study	Ref. 35

Study number	*Nanoparticle type*	*Drug*	*Indication*	*Study Phase*	*References*
		(PET) scan	tumours		
NCT03020017	Gold	NU-0129	Gliosarcoma, recurrent glioblastoma	Early Phase I	Ref. 35
NCT00734682	Liposome	CPT-11	Recurrent high-grade gliomas	Phase I	Ref. 35

Theranostics

Imaging agents and drugs transported by a single nanoparticle is another area of innovation that has been applied to the treatment of experimental brain tumours and these are known as theranostics [17,36]. Intravenously administered polymeric nanoparticles loaded with smaller iron oxide nanoparticles (for magnetic resonance imaging – MRI), surface decorated with a tumour vasculature targeting F3 peptide (a 31-amino acid sequence of the NH2-terminal fragment of human high-mobility group protein 2) and encapsulating photofrin for photodynamic therapy (PDT), were accumulated within the intracranial tumour in a 9L glioma rat model, following intravenous administration, as visualised using MRI [37]. This theranostic improved survival rates in this model following PDT when compared to plain nanoparticles in combination with PDT or photofrin alone in combination with PDT. Iron oxide as an MRI imaging agent is the contrast enhancement agent of choice with a number of theranostics. The combination of a tumour homing peptide (CGKRK), which targets the tumour endothelial and tumour cells and specifically their mitochondria with a pro-apoptotic peptide ($_{D}$[KLAKLAK]2) as the drug, when coupled to elongated iron oxide nanoparticles (nanoworms), as the MRI contrast agent, has been shown to accumulate these targeted nanoworms in the tumour tissue following intravenous injection [38]. The targeted nanoworms were significantly more effective than non-targeted nanoworms in a lentiviral (H-RasV12-shp53) induced mouse brain tumour model.

An alternative method of labelling nanoparticles for imaging in a theranostic platform involves the use of porphyrin for near infrared imaging and as such 30 nm porphyrin-lipid

apolipoprotein E3 (apoE3) lipid nanoparticles (pyE-LNs) with intrinsic imaging properties via the porphyrin lipid have been studied [39]. Across BBB delivery and tumour cell uptake properties were achieved using ApoE as ApoE is taken up by the low-density lipoprotein receptor (LDLR) on brain endothelial cells and tumour cells, where in the latter case, the LDLR receptor is upregulated [39]. After intravenous administration to a U87 Green Fluorescent Protein (GFP) mouse model, the particles were found to accumulate within brain tumour tissue.

These image competent nanotherapeutics may prove interesting in the treatment of diffuse brain metastasis in multiple brain regions.

Conclusions

Glioblastoma and brain metastasis are still areas of unmet medical need and several nanoparticle formulations are showing promise in glioblastoma rodent models of the disease with a few even transitioning to clinical testing. The leaky vasculature in brain tumours has been exploited to concentrate drug-laden nanoparticles at the tumour site, following intravenous injection. Additionally, various across BBB transport and cell uptake ligands have been employed within a single nanoparticle to enable drug to be concentrated in tumour cells in the presence of an intact BBB, following intravenous injection. These combined systems are known as dual targeting systems. Recent studies have introduced MRI and near infrared imaging to drug loaded nanoparticles, enabling targeting to be imaged with these new theranostics. The transferrin receptor has been widely exploited for across BBB transport, in these experimental studies, and a number of cell uptake ligands employed in the dual targeting approaches. It remains to be seen if the promising rodent data is indeed translatable to the clinical situation and attention will need to be turned to the issue of manufacturability if the ligand targeting systems are to transition into clinical products.

Financial & competing interests disclosure

The authors have no competing interests to declare.

References

1. Nayak L, Lee EQ, Wen PY. Epidemiology of brain metastases. *Current oncology reports*, 14(1), 48-54 (2012).
2. Kemmerer E, Shah S. Update on Radiotherapy for Central Nervous System Malignancies. *Surgical Oncology Clinics of North America*, 26(3), 347-355 (2017).
3. Sperduto PW, Kased N, Roberge D *et al.* Summary report on the graded prognostic assessment: an accurate and facile diagnosis-specific tool to estimate survival for patients with brain metastases. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, 30(4), 419-425 (2012).
4. Lutterbach J, Bartelt S, Ostertag C. Long-term survival in patients with brain metastases. *J Cancer Res Clin Oncol*, 128(8), 417-425 (2002).
5. Hardesty DA, Nakaji P. The Current and Future Treatment of Brain Metastases. *Frontiers in Surgery*, 3(30) (2016).
6. Iacob G, Dinca EB. Current data and strategy in glioblastoma multiforme. *J Med Life*, 2(4), 386-393 (2009).
7. Furnari FB, Fenton T, Bachoo RM *et al.* Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes Dev*, 21(21), 2683-2710 (2007).
8. Krex D, Klink B, Hartmann C *et al.* Long-term survival with glioblastoma multiforme. *Brain*, 130(Pt 10), 2596-2606 (2007).
9. Urbanska K, Sokolowska J, Szmidt M, Sysa P. Glioblastoma multiforme - an overview. *Contemp Oncol (Pozn)*, 18(5), 307-312 (2014).
10. Agarwal S, Manchanda P, Vogelbaum MA, Ohlfest JR, Elmquist WF. Function of the blood-brain barrier and restriction of drug delivery to invasive glioma cells: findings in an orthotopic rat xenograft model of glioma. *Drug metabolism and disposition: the biological fate of chemicals*, 41(1), 33-39 (2013).
11. Stupp R, Mason WP, van den Bent MJ *et al.* Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. *New England Journal of Medicine*, 352(10), 987-996 (2005).
12. Weller M, Cloughesy T, Perry JR, Wick W. Standards of care for treatment of recurrent glioblastoma--are we there yet? *Neuro-Oncology*, 15(1), 4-27 (2013).
13. Wong ET, Hess KR, Gleason MJ *et al.* Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, 17(8), 2572-2578 (1999).
14. J. K. Issues in Developing Drugs for Primary Brain Tumors: Barriers and Toxicities. *Toxicologic pathology*, 39(1), 152-157 (2011).
15. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci*, 7(1), 41-53 (2006).
16. Agarwal S, Sane R, Oberoi R, Ohlfest JR, Elmquist WF. Delivery of molecularly targeted therapy to malignant glioma, a disease of the whole brain. *Expert reviews in molecular medicine*, 13, e17 (2011).
17. Davis ME, Chen Z, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat Rev Drug Discov*, 7(9), 771-782 (2008).
18. Drbohlavova J, Chomoucka J, Adam V *et al.* Nanocarriers for anticancer drugs--new trends in nanomedicine. *Current drug metabolism*, 14(5), 547-564 (2013).
19. Bhojani MS, Van Dort M, Rehemtulla A, Ross BD. Targeted imaging and therapy of brain cancer using theranostic nanoparticles. *Molecular pharmaceutics*, 7(6), 1921-1929 (2010).

20. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumour vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J. Controlled Release*, 65, 271-284 (2000).
21. Dhermain FG, Hau P, Lanfermann H, Jacobs AH, van den Bent MJ. Advanced MRI and PET imaging for assessment of treatment response in patients with gliomas. *The Lancet. Neurology*, 9(9), 906-920 (2010).
22. van Tellingen O, Yetkin-Arik B, de Gooijer MC, Wesseling P, Wurdinger T, de Vries HE. Overcoming the blood-brain tumor barrier for effective glioblastoma treatment. *Drug Resist Updat*, 19, 1-12 (2015).
23. Uchegbu IF, Double JA, Kelland LR, Turton JA, Florence AT. The activity of doxorubicin niosomes against an ovarian cancer cell line and three in vivo mouse tumour models. *J Drug Target*, 3(5), 399-409 (1996).
24. Zhang Y, Yu J, Zhang L, Cai J, Cai D, Lv C. Enhanced anti-tumor effects of doxorubicin on glioma by entrapping in polybutylcyanoacrylate nanoparticles. *Tumor Biology*, 37(2), 2703-2708 (2016).
25. Wohlfart S, Khalansky AS, Gelperina S *et al.* Efficient Chemotherapy of Rat Glioblastoma Using Doxorubicin-Loaded PLGA Nanoparticles with Different Stabilizers. *PLOS ONE*, 6(5), e19121 (2011).
26. Qian L, Zheng J, Wang K *et al.* Cationic core-shell nanoparticles with carmustine contained within O(6)-benzylguanine shell for glioma therapy. *Biomaterials*, 34(35), 8968-8978 (2013).
27. Fisusi FA, Siew A, Chooi KW *et al.* Lomustine Nanoparticles Enable Both Bone Marrow Sparing and High Brain Drug Levels – A Strategy for Brain Cancer Treatments. *Pharmaceutical research*, 33(5), 1289-1303 (2016).
28. Karim R, Palazzo C, Evrard B, Piel G. Nanocarriers for the treatment of glioblastoma multiforme: Current state-of-the-art. *Journal of Controlled Release*, 227, 23-37 (2016).
29. Zhang P, Hu L, Yin Q, Feng L, Li Y. Transferrin-modified c[RGDfK]-paclitaxel loaded hybrid micelle for sequential blood-brain barrier penetration and glioma targeting therapy. *Molecular pharmaceutics*, 9(6), 1590-1598 (2012).
30. Jiang X, Xin H, Ren Q *et al.* Nanoparticles of 2-deoxy-D-glucose functionalized poly(ethylene glycol)-co-poly(trimethylene carbonate) for dual-targeted drug delivery in glioma treatment. *Biomaterials*, 35(1), 518-529 (2014).
31. Wei L, Guo XY, Yang T, Yu MZ, Chen DW, Wang JC. Brain tumor-targeted therapy by systemic delivery of siRNA with Transferrin receptor-mediated core-shell nanoparticles. *International journal of pharmaceutics*, 510(1), 394-405 (2016).
32. Zong T, Mei L, Gao H *et al.* Enhanced glioma targeting and penetration by dual-targeting liposome co-modified with T7 and TAT. *Journal of pharmaceutical sciences*, 103(12), 3891-3901 (2014).
33. Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ, Moens AL. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. *J Mol Cell Cardiol*, 52(6), 1213-1225 (2012).
34. Anselmo AC, Mitragotri S. Nanoparticles in the clinic. *Bioengineering & Translational Medicine*, 1(1), 10-29 (2016).
35. Medicine USlo. https://clinicaltrials.gov/.).
36. Meyers JD, Doane T, Burda C, Basilion JP. Nanoparticles for imaging and treating brain cancer. *Nanomedicine (London, England)*, 8(1), 123-143 (2013).
37. Reddy GR, Bhojani MS, McConville P *et al.* Vascular Targeted Nanoparticles for Imaging and Treatment of Brain Tumors. *Clinical Cancer Research*, 12(22), 6677-6686 (2006).

38. Agemy L, Friedmann-Morvinski D, Kotamraju VR *et al.* Targeted nanoparticle enhanced proapoptotic peptide as potential therapy for glioblastoma. *Proceedings of the National Academy of Sciences of the United States of America*, 108(42), 17450-17455 (2011).
39. Rajora MA, Ding L, Valic M *et al.* Tailored theranostic apolipoprotein E3 porphyrin-lipid nanoparticles target glioblastoma †Electronic supplementary information (ESI) available. See DOI: 10.1039/c7sc00732a Click here for additional data file. *Chemical Science*, 8(8), 5371-5384 (2017).

Table 1: Clinical studies on intravenously injected nanoparticles in brain cancer

Study number	***Nanoparticle type***	***Drug***	***Indication***	***Study Phase***	***References***
NCT02340156	Cationic liposomes	Liposomes encapsulated p53 cDNA in combination with oral temozolomide	Recurrent Glioblastoma	Phase II	Ref. 35
NCT02820454	Polymer-gadolinium chelates	AGuIX (polysiloxane gadolinium-chelates based nanoparticles) concurrently with whole brain radiation.	Brain metastases	Phase I	Ref. 35
NCT01266096	Silica	124I-cRGDY-PEG-dots for positron emission tomography (PET) scan	Newly diagnosed or recurrent metastatic melanoma, malignant brain tumours	Microdosing study	Ref. 35
NCT03020017	Gold	NU-0129	Gliosarcoma, recurrent glioblastoma	Early Phase I	Ref. 35
NCT00734682	Liposome	CPT-11	Recurrent	Phase I	Ref. 35

Study number	*Nanoparticle type*	*Drug*	*Indication*	*Study Phase*	*References*
			high-grade gliomas		

Keywords

Brain tumour, nanomedicine, nanoparticle, theranostic, glioblastoma, doxorubicin, paclitaxel

Brain metastases and primary CNS tumours

Brain metastases are the most frequently occurring neurologic complications of cancer in adults, with 9 – 17% of all cancers resulting in brain metastasis and brain metastasis occurring in 8 – 14 per 100,000 in the general population [1]. Primary brain tumours, on the other hand, are relatively rare, and comprise about 1.4% of cancers [2]. Brain metastases are associated with a median survival times of about 3 – 25 months [3], and a 5 year survival rate of 1.8% [4]. Treatment modalities employed for brain metastases include: surgical resection, whole brain radiation therapy, radiosurgery and chemotherapy [5]. The choice of treatment would usually be based on several considerations. These include: histopathology of the primary tumour, status of systemic disease, patient's performance status (general well being and lifestyle activity level), age of the patient, number and sites and precise location of the brain metastases (such as proximity to sites of vital brain function), co-existing morbidities, and symptoms [2,5].

Glioblastoma multiforme (WHO Classification astrocytoma Grade IV), a metastatic primary brain tumour, accounts for 12 – 15% of all brain tumours [6] and is the most common primary brain tumour in adults [7]. Glioblastoma is an aggressive metastatic astrocytoma with a median survival of 14 months and less than 5% of patients survive for 3 years [8]. This tumour is difficult to diagnose early as the tumour is usually asymptomatic or presents with symptoms which are difficult to associate with GBM, e.g. symptoms associated with a high intracranial pressure (headaches, nausea, vomiting and cognitive impairment) [9]. A major contribution to the poor survival rates is the insufficient transport of therapeutic molecules across the blood brain barrier (BBB) [10]. The current standard of care comprises surgical

resection to the maximum possible extent, followed by concurrent radio–chemotherapy and adjuvant chemotherapy with temozolomide [2]. This treatment regimen became the standard of care for newly diagnosed glioblastoma patients after the results of the 2004 European Organisation for Research and Treatment of Cancer 26981-22981/ National Cancer Institute of Canada Clinical Trials Group CE3 randomised phase III trial demonstrated a 20.7% improvement in the median survival as well as 27.2% two-year survival rates in glioblastoma patients, who had received post-surgical concomitant and adjuvant temozolomide (known as the Stupp regimen) compared to 10.9% two-year survival rates with post-surgical radiotherapy alone [11]. For recurrent glioblastoma on the other hand, there is currently no standard treatment regimen [12], and thus patients frequently receive investigational agents in clinical trials [13].

The Blood Brain Barrier (BBB)

The treatment of brain tumours (or more generally, central nervous system (CNS) tumours) is particularly challenging, mainly because of their intracranial location [14]. Intracranial tumours are effectively "shielded" from the effects of most systemically administered cytotoxic agents. The brain parenchyma and most (but not all) intracranial tumours are protected by the intact blood brain barrier (BBB), which maintains the brain microenvironment by serving as a physical and metabolic barrier regulating the access of molecules to the brain [15]. The physical barrier is formed by the tight junctions between the adjacent endothelial cells (which prevent blood-borne substances from crossing into the brain parenchyma), a lack of capillary fenestrations, very low pinocytotic activity and the metabolic barrier is formed by degradative enzymes, specialised transport receptors and endothelial cell efflux pumps [15].

Other Brain Tumour Treatment Barriers

Another barrier thought to restrict access of systemically administered therapeutic agents to tumour cells is the brain tumour-cell barrier (BTB, a barrier due to the efflux activity of tumour cells) [16]. Other challenges associated with effective brain tumour treatment are: dose limiting toxicity, mainly myelosuppression and tumour resistance to alkylating agents; the latter mediated mainly by the overexpression of O^6-methylguanine-DNA-methyltransferase (MGMT), a ubiquitous protein encoded by the MGMT gene [14].

Passive Targeting with Nanoparticles

Nanoparticles have been used to passively target drugs to intracranial tumours, on intravenous injection, in order to enable delivery of therapeutics across the BBB to the brain, as there is evidence that nanoparticles are able to preferentially accumulate drug at tumour sites, when compared to the administration of drugs in solution [17]. Generally nanoparticles may be engineered to: a) enable tissue or organ specific transport of their drug payload, or b) enable the delivery of hydrophobic and metabolically labile drugs [18,19]. Thus, nanoparticles are an interesting platform to consider in drug development for brain tumour indications [19].

Intravenously administered nanoparticles for delivery of therapeutic agents to brain tumours may theoretically exploit the enhanced permeability and retention (EPR) effect, whereby particles extravasate through a leaky tumour vasculature and achieve closer proximity to the tumour cells [20]. However for the EPR effect to be operational, the BBB must be compromised at the site of the intracranial tumour and while the breakdown of the BBB is diagnostic of a high grade glioma [21] most tumours are associated with an intact BBB [22] and direct evidence of nanoparticle accumulation within intracranial tumour cells is difficult to find. Early activity in this area focused on the delivery of P-gp efflux pump substrates to the brain in an attempt to circumvent the blood tumour-cell barrier. The P-gp substrate [23], doxorubicin, when intravenously injected in poly(butylcyanoacrylate) nanoparticles, resulted in increased tumour tissue accumulation, in a mouse C6 glioma rat model, when compared to healthy tissue and an attendant improvement in tumouricidal activity was observed with these nanoparticles when compared to the drug in solution [24]. Additionally, the formulation was

also found to be less cardiotoxic. This provides indirect evidence that nanoparticles are able to take advantage of a variation in the BBB at the tumour site.

As well as the cyanoacrylates, other polymers have also demonstrated the tumour tissue drug accumulation phenomenon on intravenous injection. Doxorubicin loaded on to poloxamer 188-coated poly-(lactic acid-co-glycolic acid) (PLGA) nanoparticles in a rat glioblastoma 101/8 model resulted in superior tumouricidal activity, through the intravenous route, when compared to the drug in solution [25].

Nanomedicines may also consist of more than one therapeutic for the treatment of brain tumours. For example, chitosan surface modified PLGA nanoparticles loaded with carmustine along with O^6-benzylguanine (which depletes MGMT, thus improving the therapeutic efficacy of carmustine). On intravenous injection, this nanoparticle formulation yielded superior survival outcomes in F98 glioma-bearing rats compared to the administration of the two drugs separately in solution or to the nanoparticle containing carmustine alone [26].

Nanoparticles may also work by simply increasing plasma exposure, which in turn increases brain exposure, while minimising exposure to areas of potential toxicity [27]. We have shown that lomustine loaded on to GCPQ (N-palmitoyl-N-monomethyl-N,N-dimethyl-N,N,N-trimethyl-6-O-glycolchitosan) nanoparticles resulted in increased plasma and brain exposure, reduced liver and bone exposure and ultimately increased tumouricidal activity (survival and tumour size) in a U87MG intracranial tumour model, without increasing myelosuppression [27].

Active Targeting with Nanoparticles

Active targeting involves the use of carriers bearing various surface ligands to achieve either transport across an intact BBB, or alternatively, cell uptake following extravasation across a leaky BBB [28].

Various across BBB transporters have been exploited for transport across an intact BBB, such as the transferrin receptor [29] and the GLUT receptor [30]. Transferrin – cyclo-[Arg-Gly-Asp-d-Phe-Lys] - c[RGDfK] paclitaxel micelles have been prepared and injected intravenously to a U87MG mouse model, with transferrin included to enable across BBB transport while c[RGDfK] was included to enable uptake by tumour cells [29]. This resulted in drug accumulation in the brain and a superior anti-glioma effect compared to the commercial formulation, Taxol [29]. Others have utilised the T7 peptide (HAIYPRH) to

target the endothelial cell transferrin receptor and achieve across BBB transport [31]. T7 peptide modified core-shell nanoparticles (T7-LPC/siRNA) have been shown, on intravenous administration, to accumulate anti-epidermal growth factor receptor (anti-EGFR) siRNA in intracranial tumour tissue, down regulate EGFR and increase survival rates in a U87MG mouse tumour model, when compared to plain nanoparticles [31]. While the T7 peptide appears to achieve delivery across the BBB [31], efforts to improve cell uptake, once extravasation has taken place, have involved the use of dual targeting strategies, in which transport across the BBB is combined with a ligand promoting tumour cell uptake [32]. Intravenously administered dual targeted doxorubicin liposomes comprising the TAT peptide (AYGRKKRRQRRR) for cellular uptake and the T7 peptide for across BBB transport resulted in increased delivery of doxorubicin to the brain glioma tissue in a C6 glioma mouse model and reduced delivery to the heart, which is relevant for the cardiotoxic [33] drug doxorubicin [32].

Utilisation of the GLUT receptor to cross the BBB has been achieved by using 2-deoxy-D-glucose modified poly(ethylene glycol)-co-poly(trimethylene carbonate) paclitaxel nanoparticles [30]. The 2-deoxy-D-glucose moiety was correlated with drug accumulation in the brain and these glucose-decorated nanoparticles produced superior survival in an RG2 mouse glioma model, when compared to plain nanoparticles and Taxol [30].

While there is good preclinical evidence showing the efficacy of nanoparticles in rodent models of intracranial tumours, clinical evidence on the use of nanoparticles is harder to locate. There are some reports of clinical trials in brain tumour patients with passively targeted nanoparticles (Table 1): e.g. NCT02340156, NCT02820454, NCT01266096, NCT03020017, NCT00734682 [34,35], however the efficacy of this nanoparticle approach in the clinic has not yet been reported.

Table 1: Clinical studies on intravenously injected nanoparticles in brain cancer

Study number	***Nanoparticle type***	***Drug***	***Indication***	***Study Phase***	***References***
NCT02340156	Cationic liposomes	Liposomes encapsulated p53 cDNA in combination with oral	Recurrent Glioblastoma	Phase II	Ref. 35

Study number	*Nanoparticle type*	*Drug*	*Indication*	*Study Phase*	*References*
		temozolomide			
NCT02820454	Polymer-gadolinium chelates	AGuIX (polysiloxane gadolinium-chelates based nanoparticles) concurrently with whole brain radiation.	Brain metastases	Phase I	Ref. 35
NCT01266096	Silica	124I-cRGDY-PEG-dots for positron emission tomography (PET) scan	Newly diagnosed or recurrent metastatic melanoma, malignant brain tumours	Microdosing study	Ref. 35
NCT03020017	Gold	NU-0129	Gliosarcoma, recurrent glioblastoma	Early Phase I	Ref. 35
NCT00734682	Liposome	CPT-11	Recurrent high-grade gliomas	Phase I	Ref. 35

Theranostics

Imaging agents and drugs transported by a single nanoparticle is another area of innovation that has been applied to the treatment of experimental brain tumours and these are known as theranostics [17,36]. Intravenously administered polymeric nanoparticles loaded with smaller iron oxide nanoparticles (for magnetic resonance imaging – MRI), surface decorated with a tumour vasculature targeting F3 peptide (a 31-amino acid sequence of the NH2-terminal fragment of human high-mobility group protein 2) and encapsulating photofrin for photodynamic therapy (PDT), were accumulated within the intracranial tumour in a 9L glioma rat model, following intravenous administration, as visualised using MRI [37]. This theranostic improved survival rates in this model following PDT when compared to plain nanoparticles in combination with PDT or photofrin alone in combination with PDT. Iron oxide as an MRI imaging agent is the contrast enhancement agent of choice with a number of theranostics. The combination of a tumour homing peptide (CGKRK), which targets the tumour endothelial and tumour cells and specifically their mitochondria with a pro-apoptotic peptide ($_{D}$[KLAKLAK]2) as the drug, when coupled to elongated iron oxide nanoparticles (nanoworms), as the MRI contrast agent, has been shown to accumulate these targeted nanoworms in the tumour tissue following intravenous injection [38]. The targeted nanoworms were significantly more effective than non-targeted nanoworms in a lentiviral (H-RasV12-shp53) induced mouse brain tumour model.

An alternative method of labelling nanoparticles for imaging in a theranostic platform involves the use of porphyrin for near infrared imaging and as such 30 nm porphyrin-lipid apolipoprotein E3 (apoE3) lipid nanoparticles (pyE-LNs) with intrinsic imaging properties via the porphyrin lipid have been studied [39]. Across BBB delivery and tumour cell uptake properties were achieved using ApoE as ApoE is taken up by the low-density lipoprotein receptor (LDLR) on brain endothelial cells and tumour cells, where in the latter case, the LDLR receptor is upregulated [39]. After intravenous administration to a U87 Green Fluorescent Protein (GFP) mouse model, the particles were found to accumulate within brain tumour tissue.

These image competent nanotherapeutics may prove interesting in the treatment of diffuse brain metastasis in multiple brain regions.

Conclusions

Glioblastoma and brain metastasis are still areas of unmet medical need and several nanoparticle formulations are showing promise in glioblastoma rodent models of the disease with a few even transitioning to clinical testing. The leaky vasculature in brain tumours has been exploited to concentrate drug-laden nanoparticles at the tumour site, following intravenous injection. Additionally, various across BBB transport and cell uptake ligands have been employed within a single nanoparticle to enable drug to be concentrated in tumour cells in the presence of an intact BBB, following intravenous injection. These combined systems are known as dual targeting systems. Recent studies have introduced MRI and near infrared imaging to drug loaded nanoparticles, enabling targeting to be imaged with these new theranostics. The transferrin receptor has been widely exploited for across BBB transport, in these experimental studies, and a number of cell uptake ligands employed in the dual targeting approaches. It remains to be seen if the promising rodent data is indeed translatable to the clinical situation and attention will need to be turned to the issue of manufacturability if the ligand targeting systems are to transition into clinical products.

Financial & competing interests disclosure

The authors have no competing interests to declare.

References

1. Nayak L, Lee EQ, Wen PY. Epidemiology of brain metastases. *Current oncology reports*, 14(1), 48-54 (2012).
2. Kemmerer E, Shah S. Update on Radiotherapy for Central Nervous System Malignancies. *Surgical Oncology Clinics of North America*, 26(3), 347-355 (2017).
3. Sperduto PW, Kased N, Roberge D *et al.* Summary report on the graded prognostic assessment: an accurate and facile diagnosis-specific tool to estimate survival for patients with brain metastases. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, 30(4), 419-425 (2012).
4. Lutterbach J, Bartelt S, Ostertag C. Long-term survival in patients with brain metastases. *J Cancer Res Clin Oncol*, 128(8), 417-425 (2002).

5. Hardesty DA, Nakaji P. The Current and Future Treatment of Brain Metastases. *Frontiers in Surgery*, 3(30) (2016).
6. Iacob G, Dinca EB. Current data and strategy in glioblastoma multiforme. *J Med Life*, 2(4), 386-393 (2009).
7. Furnari FB, Fenton T, Bachoo RM *et al.* Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes Dev*, 21(21), 2683-2710 (2007).
8. Krex D, Klink B, Hartmann C *et al.* Long-term survival with glioblastoma multiforme. *Brain*, 130(Pt 10), 2596-2606 (2007).
9. Urbanska K, Sokolowska J, Szmidt M, Sysa P. Glioblastoma multiforme - an overview. *Contemp Oncol (Pozn)*, 18(5), 307-312 (2014).
10. Agarwal S, Manchanda P, Vogelbaum MA, Ohlfest JR, Elmquist WF. Function of the blood-brain barrier and restriction of drug delivery to invasive glioma cells: findings in an orthotopic rat xenograft model of glioma. *Drug metabolism and disposition: the biological fate of chemicals*, 41(1), 33-39 (2013).
11. Stupp R, Mason WP, van den Bent MJ *et al.* Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. *New England Journal of Medicine*, 352(10), 987-996 (2005).
12. Weller M, Cloughesy T, Perry JR, Wick W. Standards of care for treatment of recurrent glioblastoma--are we there yet? *Neuro-Oncology*, 15(1), 4-27 (2013).
13. Wong ET, Hess KR, Gleason MJ *et al.* Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, 17(8), 2572-2578 (1999).
14. J. R. Issues in Developing Drugs for Primary Brain Tumors: Barriers and Toxicities. *Toxicologic pathology*, 39(1), 152-157 (2011).
15. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci*, 7(1), 41-53 (2006).
16. Agarwal S, Sane R, Oberoi R, Ohlfest JR, Elmquist WF. Delivery of molecularly targeted therapy to malignant glioma, a disease of the whole brain. *Expert reviews in molecular medicine*, 13, e17 (2011).
17. Davis ME, Chen Z, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat Rev Drug Discov*, 7(9), 771-782 (2008).
18. Drbohlavova J, Chomoucka J, Adam V *et al.* Nanocarriers for anticancer drugs--new trends in nanomedicine. *Current drug metabolism*, 14(5), 547-564 (2013).
19. Bhojani MS, Van Dort M, Rehemtulla A, Ross BD. Targeted imaging and therapy of brain cancer using theranostic nanoparticles. *Molecular pharmaceutics*, 7(6), 1921-1929 (2010).
20. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumour vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J. Controlled Release*, 65, 271-284 (2000).
21. Dhermain FG, Hau P, Lanfermann H, Jacobs AH, van den Bent MJ. Advanced MRI and PET imaging for assessment of treatment response in patients with gliomas. *The Lancet. Neurology*, 9(9), 906-920 (2010).
22. van Tellingen O, Yetkin-Arik B, de Gooijer MC, Wesseling P, Wurdinger T, de Vries HE. Overcoming the blood-brain tumor barrier for effective glioblastoma treatment. *Drug Resist Updat*, 19, 1-12 (2015).
23. Uchegbu IF, Double JA, Kelland LR, Turton JA, Florence AT. The activity of doxorubicin niosomes against an ovarian cancer cell line and three in vivo mouse tumour models. *J Drug Target*, 3(5), 399-409 (1996).

24. Zhang Y, Yu J, Zhang L, Cai J, Cai D, Lv C. Enhanced anti-tumor effects of doxorubicin on glioma by entrapping in polybutylcyanoacrylate nanoparticles. *Tumor Biology*, 37(2), 2703-2708 (2016).
25. Wohlfart S, Khalansky AS, Gelperina S *et al.* Efficient Chemotherapy of Rat Glioblastoma Using Doxorubicin-Loaded PLGA Nanoparticles with Different Stabilizers. *PLOS ONE*, 6(5), e19121 (2011).
26. Qian L, Zheng J, Wang K *et al.* Cationic core-shell nanoparticles with carmustine contained within O(6)-benzylguanine shell for glioma therapy. *Biomaterials*, 34(35), 8968-8978 (2013).
27. Fisusi FA, Siew A, Chooi KW *et al.* Lomustine Nanoparticles Enable Both Bone Marrow Sparing and High Brain Drug Levels – A Strategy for Brain Cancer Treatments. *Pharmaceutical research*, 33(5), 1289-1303 (2016).
28. Karim R, Palazzo C, Evrard B, Piel G. Nanocarriers for the treatment of glioblastoma multiforme: Current state-of-the-art. *Journal of Controlled Release*, 227, 23-37 (2016).
29. Zhang P, Hu L, Yin Q, Feng L, Li Y. Transferrin-modified c[RGDfK]-paclitaxel loaded hybrid micelle for sequential blood-brain barrier penetration and glioma targeting therapy. *Molecular pharmaceutics*, 9(6), 1590-1598 (2012).
30. Jiang X, Xin H, Ren Q *et al.* Nanoparticles of 2-deoxy-D-glucose functionalized poly(ethylene glycol)-co-poly(trimethylene carbonate) for dual-targeted drug delivery in glioma treatment. *Biomaterials*, 35(1), 518-529 (2014).
31. Wei L, Guo XY, Yang T, Yu MZ, Chen DW, Wang JC. Brain tumor-targeted therapy by systemic delivery of siRNA with Transferrin receptor-mediated core-shell nanoparticles. *International journal of pharmaceutics*, 510(1), 394-405 (2016).
32. Zong T, Mei L, Gao H *et al.* Enhanced glioma targeting and penetration by dual-targeting liposome co-modified with T7 and TAT. *Journal of pharmaceutical sciences*, 103(12), 3891-3901 (2014).
33. Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ, Moens AL. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. *J Mol Cell Cardiol*, 52(6), 1213-1225 (2012).
34. Anselmo AC, Mitragotri S. Nanoparticles in the clinic. *Bioengineering & Translational Medicine*, 1(1), 10-29 (2016).
35. Medicine USlo. https://clinicaltrials.gov/.).
36. Meyers JD, Doane T, Burda C, Basilion JP. Nanoparticles for imaging and treating brain cancer. *Nanomedicine (London, England)*, 8(1), 123-143 (2013).
37. Reddy GR, Bhojani MS, McConville P *et al.* Vascular Targeted Nanoparticles for Imaging and Treatment of Brain Tumors. *Clinical Cancer Research*, 12(22), 6677-6686 (2006).
38. Agemy L, Friedmann-Morvinski D, Kotamraju VR *et al.* Targeted nanoparticle enhanced proapoptotic peptide as potential therapy for glioblastoma. *Proceedings of the National Academy of Sciences of the United States of America*, 108(42), 17450-17455 (2011).
39. Rajora MA, Ding L, Valic M *et al.* Tailored theranostic apolipoprotein E3 porphyrin-lipid nanoparticles target glioblastoma †Electronic supplementary information (ESI) available. See DOI: 10.1039/c7sc00732a Click here for additional data file. *Chemical Science*, 8(8), 5371-5384 (2017).