

# **Realising the therapeutic potential of rare earth elements in designing nanoparticles to target and treat glioblastoma**

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**Abstract:**

The prognosis of brain cancer glioblastoma is poor, and despite intense research, there have been no significant improvements within the last decade. This stasis implicates the need for more novel therapeutic investigation. One such option is the use of nanoparticles, which can be beneficial due to their ability to penetrate the brain, overcome the blood brain barrier and take advantage of the enhanced permeation and retention effect of glioblastoma to improve specificity. Rare earth elements possess a number of interesting natural properties due to their unique electronic configuration which may prove therapeutically advantageous in a nanoparticle formulation. The under-explored potential for rare earth elements to augment the therapeutic potential of nanoparticles in glioblastoma treatment is discussed in this review.

**Keywords:** Glioblastoma, nanoparticle, rare earth oxide, blood brain barrier, cytotoxicity, radiosensitisation, cancer.

## 1. Glioblastoma

Glioblastoma (GBM; Grade IV astrocytoma) are tumours of the brain believed to arise from astrocyte progenitor cells, and account for 15% of all primary brain cancers.[1, 2] Current conventional treatment involves neurosurgical resection followed by concurrent radiation therapy and chemotherapy.[3, 4] However, even with optimal treatment, the expected survival time is 12 to 15 months, and only 3 months without any treatment.[5] Furthermore, in those cases where initial treatment is successful, there is a high likelihood of recurrence.

### *1.1 The astrocyte model*

To date, GBM have been categorised as astrocytic in origin due to their morphological and lineage marker similarities to that of normal and reactive astrocytes.[6] This model is the most common cellular model used to conceptualize GBM, and will be referred to as the *astrocyte model* in this review. However, it is still uncertain if GBM cells necessarily originate from the same glial progenitors as astrocytes do, such as neural stem cells, or if the cellular characteristics of one can be directly attributed for the other just yet.[7] One current perspective is that because these astrocyte progenitor cells persist and self-renew into adulthood, they may be the origin of malignant cellular transformation seen in GBM after aberrant differentiation into what should have been astrocyte cells.[8] Transcriptomic and genomic studies have shown that there are many distinct differentiation points along the pathway to astrocyte development that can result in different GBM subtypes.[9]

## 2. Rare earth element nanoparticles and the brain

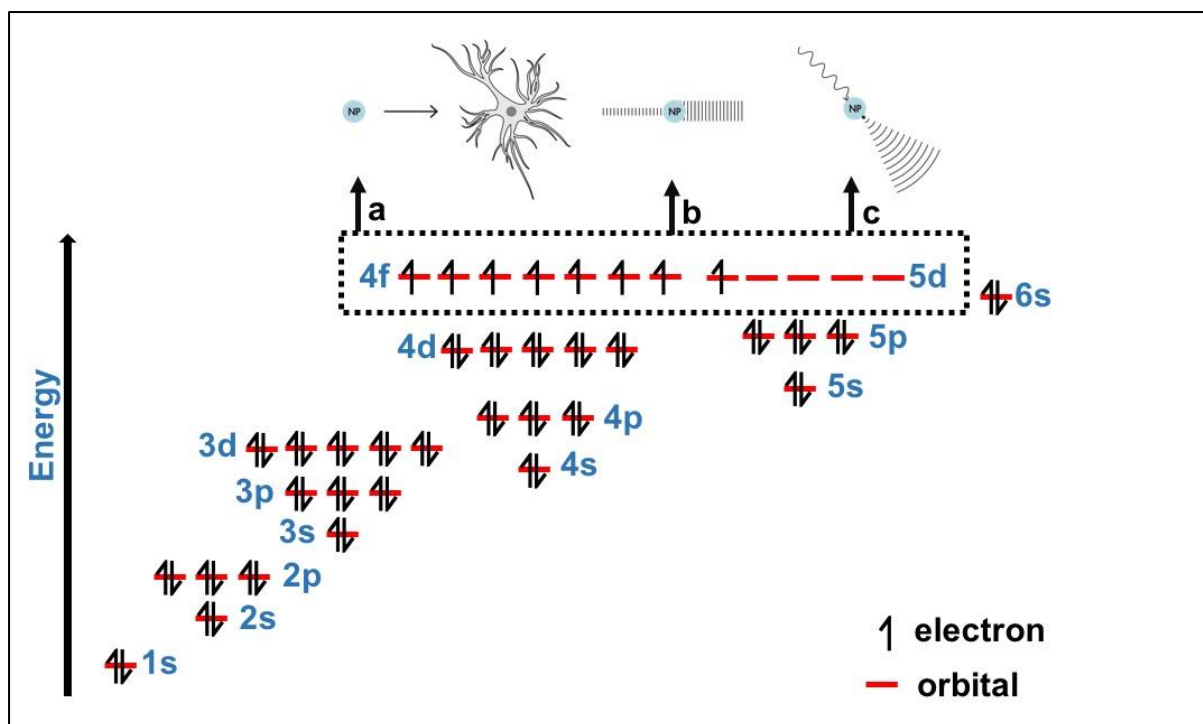
The rare earth elements (REEs) are a series of non-radioactive elements that consist of seventeen *f*-electropositive metals; lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu). Scandium (Sc) and yttrium (Y) are also considered REEs because of similar chemical and toxicological characteristics. Despite their name, REEs are not especially rare, and are readily found in the Earth's crust as oxides. REEs can be found naturally within the human body, with average intravenous levels in the order of 1 ng/kg.[10] Such levels are 1000 fold lower than the LD<sub>50</sub> (median lethal dose) of 1 mg/kg for levels in mice.[11, 12]

All REEs exhibit preference for the oxidation state +3 with an unprecedented uniformity amongst other chemical series in the periodic table. This is attributed to the core-like nature of their unique isolated 4*f* orbitals (Figure 1). All RE<sup>3+</sup> ions have the configuration [Xe]4*f*<sup>*n*</sup>6*s*<sup>2</sup> with the exceptions of Ce, Gd and Lu, where the 5*d* orbital is singly occupied (Table 1). The particular interest in these elements comes from the number of unpaired electrons these orbitals can hold and how they may interact biologically with GBM cells.

As a consequence of their unique electronic configurations, REEs react vigorously with O<sub>2</sub> to produce highly-stable oxides; in most cases these are the sesquioxides, (REE)<sub>2</sub>O<sub>3</sub>, but other REE with reasonably low fourth ionization energies form higher oxides, specifically CeO<sub>2</sub>, Pr<sub>6</sub>O<sub>11</sub> and Tb<sub>4</sub>O<sub>7</sub>. [13] These REE oxides can be readily formulated into nanoparticles (NPs) due to their chemical stability. The primary attraction for using NPs in the brain is their proven ability to reach and infiltrate brain matter [14-16]. Indeed, , *in vivo* animal studies [17-19] studying intravenous delivery of NPs up to 160 nm in size have shown NP presence in brain matter using transmission electron microscopy.

## 2.1 Applications in imaging

The combination of high availability, chemical stability and NP formulation of REE oxide NPs has spurred the application of this technology in the management of brain cancer. REEs are particularly noteworthy for their use as contrast agents for magnetic resonance imaging (MRI).[20] This technique is a non-radiation, high-resolution imaging modality that measures proton relaxation of water molecules after exposure to radiofrequency excitation. Paramagnetic contrast agents, those with unpaired electrons, can enhance the detection of these energy changes by extending relaxation time or augmenting magnetic moment. This makes REEs an attractive consideration as given their expanded electronic  $4f$  subshells all  $\text{RE}^{3+}$  ions possess at least one unpaired electron and are paramagnetic, with the exceptions of  $\text{La}^{3+}$  and  $\text{Lu}^{3+}$ . [21] Amongst the REEs,  $\text{Gd}^{3+}$  remains the most commonly used element owing to its seven unpaired  $4f$  electrons, large magnetic moment, and long electronic relaxation time to enhance imaging brightness.[22]  $\text{Gd}_2\text{O}_3$ -based NPs have been shown to be as effective as Gd-conjugated complexes in T1-weighted MRI.[23] In terms of brain cancer, ultra-small  $\text{Gd}_2\text{O}_3$ -based NPs (2 to 3 nm in diameter) have been successfully demonstrated to be internalized and retained in GBM cells, allowing for successful identification *in vivo* using MRI.[24] For a more in-depth analysis on REEs in NP formulation for bioimaging applications relevant to the brain there are a number of excellent reviews[25, 26] **STAR THIS**



**Figure 1. The ground state electronic configuration of typical rare earth element (REE) gadolinium.** The 4f and 5d orbitals in the dotted box represent the high energy orbitals that are unique to rare earth elements, and are filled according to Table 1. The unpaired electrons in these orbitals are believed to be involved in the anticancer properties of REEs in glioblastoma (GBM) therapy. In nanoparticle (NP) formulation, these mechanisms include a) direct cytotoxicity b) augmenting radiation therapy and c) augmenting chemotherapy.

Rare Earth Element	Ground state electronic configuration	Unpaired electrons
Lanthanum, La	[Xe] 5d <sup>1</sup> 6s <sup>2</sup>	1
Cerium, Ce	[Xe] 4f <sup>1</sup> 5d <sup>1</sup> 6s <sup>2</sup>	2
Praseodymium, Pr	[Xe] 4f <sup>3</sup> 6s <sup>2</sup>	3
Neodymium, Nd	[Xe] 4f <sup>4</sup> 6s <sup>2</sup>	4
Promethium, Pm	[Xe] 4f <sup>5</sup> 6s <sup>2</sup>	5
Samarium, Sm	[Xe] 4f <sup>6</sup> 6s <sup>2</sup>	6
Europium, Eu	[Xe] 4f <sup>7</sup> 6s <sup>2</sup>	7
Gadolinium, Gd	[Xe] 4f <sup>7</sup> 5d <sup>1</sup> 6s <sup>2</sup>	8
Terbium, Tb	[Xe] 4f <sup>9</sup> 6s <sup>2</sup>	5
Dysprosium, Dy	[Xe] 4f <sup>10</sup> 6s <sup>2</sup>	4
Holmium, Ho	[Xe] 4f <sup>11</sup> 6s <sup>2</sup>	3
Erbium, Er	[Xe] 4f <sup>12</sup> 6s <sup>2</sup>	2
Thulium, Tm	[Xe] 4f <sup>13</sup> 6s <sup>2</sup>	1
Ytterbium, Yb	[Xe] 4f <sup>14</sup> 6s <sup>2</sup>	0
Lutetium, Lu	[Xe] 4f <sup>14</sup> 5d <sup>1</sup> 6s <sup>2</sup>	1
Scandium, Sc	[Ar] 3d <sup>1</sup> 4s <sup>2</sup>	1
Yttrium, Y	[Kr] 4d <sup>1</sup> 5s <sup>2</sup>	1

**Table 1. Electronic configuration of all rare earth elements and number of unpaired electrons.**

[Xe] = [Kr] 4d<sup>10</sup> 5s<sup>2</sup> 5p<sup>6</sup>; [Kr] = [Ar] 3d<sup>10</sup> 4s<sup>2</sup> 4p<sup>6</sup>; [Ar] = 1s<sup>2</sup>2s<sup>2</sup> 2p<sup>6</sup>3s<sup>2</sup> 3p<sup>6</sup>

### 3. Mechanisms of REE NPs against glioblastoma

The emerging field of theranostics ('therapy'+ 'diagnostics') has overlapped with NP technology in the search of better management options for GBM. A general review on the application of nanoparticles is given by Yao et al., [27], whereas herein we will concentrate on the specific use of REE nanoparticles – **STAR THIS**. While there are clear benefits from using REE NPs as diagnostic tools, primarily that of imaging, the therapy aspect remains underdeveloped. Therapeutic aspects are discussed in the sections below:

#### *3.1 Inducing natural cytotoxicity*

The ionic radii of REEs ranges from 98 to 116 pm.[28] This is particularly important since the ionic radius of  $\text{Ca}^{2+}$  is 99 pm, and therefore REEs readily compete with  $\text{Ca}^{2+}$  ions that are crucial for the regulation of many proteins within astrocytes, and thus theoretically at least, in GBM cells.[29] The mobilization of internal calcium, as well as calcium entry across the plasma membrane, contribute to the ATP-evoked changes in protein phosphorylation within astrocytes.[30] These calcium-dependent phosphorylation systems are involved in a wide range of vital cellular functions, including energy metabolism and ion channel activity as well as cell motility, proliferation and differentiation.[31] More specifically, these processes include the phosphorylation of large proteins (55 and 52 kDa) by calcium-dependent protein kinases such as calmodulin-dependent protein kinase and protein kinase C, and the dephosphorylation of smaller proteins (24 and 21 kDa) by calcium-dependent protein phosphatases such as calcineurin.[32, 33] The activity of these protein phosphorylation systems in astrocytes are decreased in the presence of REEs such as La.[30] This demonstrates the potential of REEs to affect change in astrocytes at a cellular level that interrupts normal function leading to cell death.[34]

Furthermore, in each astrocyte there is an overriding tonic inhibition of excitatory glutamatergic neurotransmission triggered by successive rises in the intracellular  $\text{Ca}^{2+}$  from adjacent astrocytes.[35, 36] Any interferences in the intracellular  $\text{Ca}^{2+}$  content of astrocytes by REEs can then alleviate this



inhibition leading to an excitatory neurotoxicity and eventual death. [37] Hence there appears possible mechanisms by which the ionic radii of REEs can compete with  $\text{Ca}^{2+}$  to exert a cytotoxic effect on astrocytic cells. The reality is most likely a synergistic combination of multiple processes.

### *3.2 Improving radiation efficiency by radiosensitization*

Radiation therapy is frequently used to either reduce the size of GBM prior to surgery or as adjuvant therapy.[38] Radiation therapy works by reacting with the aqueous environment to generate toxic radical oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radicals. The ROS are unstable and highly reactive compounds that damage cellular DNA, compromise cellular repair mechanisms and trigger apoptotic processes within cells, collectively leading to cellular death.[39, 40] Various mechanisms can be employed to enhance the efficacy of radiotherapy, such as the placement of radiosensitizers at the tumor site. Due to their electronic structure, REEs display the Auger effect in response to irradiation which may provide efficient radiosensitization in GBM.

The *Auger effect* describes the release of an electron from an outer-shell to replenish an inner-shell ionized by radiation, which then leads to an energy emission.[41] In the case of the REEs with a large number of electron orbitals of differing energy, the Auger effect can repeat itself across multiple times, creating an Auger cascade which leads to the amplification of the number of electrons released and subsequent energy emitted in addition to the incoming radiation dose. Placement of REE NPs within tumors could therefore enhance the power of conventional radiotherapy.

Radiosensitization in the presence of REE Gd has been implicated both in vitro and in vivo. Using their  $\text{Gd}_2\text{O}_3$ -based NPs in the U87 cell line, Mowat et al.[42] successfully demonstrated a significant decrease in survival of cells exposed to radiation, similar to routine X-ray therapy, in the presence of the NPs. While the supporting evidence in human subjects is yet to be reported, it is promising that significantly longer survival times have been implicated in animal models. Le Duc et al.[19] evaluated radiosensitization potential of their  $\text{Gd}_2\text{O}_3$ -based NPs in GBM-inoculated rats exposed

to highly selective microbeam radiation therapy (MRT) at either 5 or 20 minutes after intravenous NP injection. The lifespan of rats treated at 20 minutes after injection was increased approximately fourfold when compared to those treated 5 minutes after injection. Presumably the time delay between the two groups allowed for greater infiltration of the tumour by the NPs in the 20-minute group, and thus a greater radiosensitization and survival effect. The main weakness of conventional radiation therapy is the lack of cancer specificity and control for sensitivity. By the Auger effect, REEs present a novel option to augment incoming radiation dose at a target site, reducing the intensity of the incoming radiation and its collateral damage to the healthy brain matter it passes to reach the GBM target.

### *3.3 Improving chemotherapy delivery by photosensitisation*

Due to the rich ladder-like energy-level patterns of REEs, they are ideal for facilitating efficient luminescence in vivo.[43] Systematic data has been collected on the absorption of REE ions in aqueous solution as early as the 1930's.[44] The absorption spectra show a systematic trend with those elements with those elements at the beginning and at the end of the REE group (except Ce and Yb) having absorption bands in the long-wavelength region of the spectrum, which gradually shift towards shorter wavelengths (than clinical X-ray, thus less radiation) with elements lying near the middle of the group.

The ability for REE NPs to augment the yield of chemotherapy is the least researched area with regards GBM management.. However, one way in which REE NPs may be utilized involved the use of up-conversion luminescence and cleavage of pro-drugs, or activation by photosensitization. [45]. *Upconversion luminescence* (UCL), otherwise known as photon upconversion or anti-Stokes emission, is the sequential absorption of two or more low-energy photons which leads to a higher-energy emission or luminescence.[46] The generation of upconversion NPs could provide an interesting class of materials which are superior to other conventional fluorophores by giving high signal-to-noise ratio, and better photostability. Excitation in the NIR region could also provide deeper tissue penetration and lower photodamage to biological samples.[47] Furthermore, NP formulations with REEs could be used to activate photosensitive chemotherapy agents coupled to their surface, and induce a local

photodynamic cytotoxicity. This concept has been demonstrated to inhibit growth of GBM in mice using the cytotoxic Chlorin e6 (Ce6) photosensitizer conjugated to Yb and Er NPs.[48] The Ce6 group also aided the passage of the REE NPs through the BBB into the GBM cells due to the lipophilic and hydrophobic nature of the photosensitizer. [49]

## 4. Designing REE nanoparticles for glioblastoma

There are a number of key parameters which need to be considered when designing NPs for use in GBM treatment; they must be able to reach the target site, infiltrate, and then undergo uptake by the cancer cells.

### 4.1 Size

To be effective, NPs must be small enough to remain in circulation to reach the GBM, however large enough to avoid excessive excretion by renal and hepatic clearance.[50] Based on these requirements, the optimal NP size for cellular uptake in cancer cells has been defined as <100 nm.[51, 52] Given that NPs need to be able to accumulate in the cancer interstitium after leaving the circulation, size is a compromise between penetration and retention when administered systemically. Perrault et al.[50] found that larger NPs >100 nm appeared to stay nearer the vasculature, permeated less and were retained for longer at the target sites when compared to smaller NPs ~20 nm which penetrated deeper, diffused more quickly throughout the interstitium, but were retained for shorter amounts of time.

Uptake across the cell membrane is both size and shape dependent, [53] and also related to the material composition. Interactions of the NPs with the membrane can therefore include electrostatic and hydrophobic forces, and uptake will be a combination of bending and stretching forces in the membrane. A study by Chithrani et al. [54] showed that NPs 50 nm in diameter exhibited the greatest overall uptake in brain cancer cells.

### 4.2 Shape

The morphology and aspect ratio of a NP will influence the probability and speed of uptake into the GBM cell. Spherical NPs have been shown to be rapidly internalised due to their symmetrical structure.[55, 56] Rod shaped NPs, on the other hand, are internalised at varying rates dependent on

their presenting axis to the cellular membrane given NP asymmetry enhances charge and ligand presentation depending on shape to influence cellular uptake.[57] This is because when uptake is initiated, the local curvature of NPs affects the degree to which they fit the contours of target cell membranes, which are rarely symmetrical.[58] Shape and size are often considered together in design, as the efficiency of a NP shape is dependent in part by its size. Chithrani et al.[59] demonstrated that NPs <100nm favored a more spherical than rod shape for uptake in cervical cancer cells. In the same cell line, Gratton et al.[60] observed greater uptake for NPs >100 nm favored rods over spheres.

#### *4.3 Surface charge*

The surface charge on NPs is also an influential parameter, as positive charge is associated with greater cellular uptake than negative or neutral charge. Indeed, Slowing et al.[61] demonstrated that in cervical cancer cell lines there was a negative linear relationship between NP surface charge and median effective dose (ED<sub>50</sub>). The greater effect exerted by positive charge is attributed to the slight negative charge of the cellular membrane and consequently, cellular uptake is driven by the attracting electrostatic interactions that bind the NPs to the cellular membrane.[62] However, the inevitable process of protein opsonisation of NPs in serum should be taken into consideration since this will likely interfere with NP-membrane interactions.[54]

## 5. Reaching glioblastoma with nanoparticles of rare earth elements

The blood vessels that transverse the brain matter, facilitating vital exposure for necessary resources to maintain the tissue, are encased in a layer of cerebral endothelial cells which collectively constitutes the blood brain barrier (BBB). The BBB regulates what can penetrate from circulation to brain matter, and serves as a physical barrier to protect the brain from toxic materials; unfortunately, this also includes most therapeutic drugs.[63] The BBB is so effective that estimates suggest more than 98% of small-molecule drugs and effectively 100% of large-molecule drugs are prevented from reaching the brain by the BBB.[64]

### 5.1 Overcoming the blood brain barrier

The primary promise of NPs to treat GBM is their proven ability to penetrate the cerebral endothelial cells of the BBB from the circulation, and to reach the GBM.[14-16] The most accepted pathway of natural NP uptake through the BBB appears to be via adsorptive transcytosis. [65] This type of transport describes a vector movement of molecules within endocytic vesicles across the cerebral endothelial cells, from the luminal (blood) side to the abluminal (brain) side, followed by exocytosis into the brain interstitium.[66] (Figure 2) The initial surface adsorption at the luminal surface by the NP depends if the process initiates from a specific or a non-specific ligand-interaction, referred to as *receptor-mediated transcytosis* (RMT) or *adsorptive-mediated transcytosis* (AMT), respectively. The three best-known ligands that facilitate the RMT of NPs through the BBB are insulin, iron-transferrin and LDL-cholesterol. [67-69] AMT on the other hand is a consequence of electrostatic charge-charge interactions between cationic macromolecules and the negative charges on the luminal surface of the BBB. At systemic physiological pH, the luminal surface of the BBB is anionic largely due to the presence of the sialic acid residues of glycoproteins.[70]

Once adsorbed, the NP interacts at the endothelial cells *via* one of two entry site types (either caveolae or clathrin-coated pits) on the luminal surface. From these sites transcytotic vesicles are

formed, which then transport the NP to the abluminal surface before releasing it into the brain interstitium. The caveolae form free intact vesicles that are directly transported across the cell whereas the clathrin-coated pits form vesicles and fuse with an intracellular endosome before being transported across the endothelial cell.[71, 72] Additionally, it should be noted that the intercellular junctions between adjacent endothelial cells of the BBB can be as small as 1 nm, which are too small for most NPs to pass through, thereby relying on adsorptive transcytosis to overcome the BBB.[73]

#### *5.1.1 Enhanced permeability and retention*

It was proposed many decades ago that solid tumours possessed unique pathophysiological traits compared to normal healthy tissue.[74] Solid tumours exhibit extensive angiogenesis and hence hypervascularity, defective vascular architecture, impaired lymphatic drainage and recovery system, and greatly increased production of a number of permeability mediators.[75] Collectively, these phenomena produce an *enhanced permeability and retention* (EPR) effect for endogenous and exogenous substances within an organ interstitium. Specific to the brain, the EPR effect has been associated with a number of changes observed in the locality of GBM. This primarily includes the breakdown of the BBB[76], the downregulation of the tight junction protein claudin creating larger intercellular junction gaps[77], as well as a redistribution of glial protein aquaporin AQP4 which disturbs the water balances within the brain[78]. Clinically, the deterioration of BBB integrity induces cerebral edema which can manifest as headaches, loss of consciousness or seizures.[79]

The EPR effect provides an enhanced avenue for NPs to better penetrate the BBB and access GBM. This is of particular significance when considering inoperable GBM cases which involve diffuse infiltration and proximity to eloquent grey matter structures. Furthermore, there will be a greater concentration of NPs in the target GBM cells themselves, and less incidental exposure to non-cancerous cells. However, this effect has been noted to be significantly less pronounced in the brain microenvironment than in cancers elsewhere in the body.[80] Consequently, optimal delivery load of NPs may be different in GBM compared to other cancers.[81]

### 5.1.2 Augmenting BBB penetration by functionalisation

Functionalisation is the process of modifying NPs by specific ligand addition to enhance their efficiency in reaching the brain. A number of ligands conjugated to NPs have been shown to improve BBB penetration, and can be broadly separated into four different groups:

- i) ligands that mediate adsorption at the BBB interface to initiate transcytosis, e.g. poly(sorbate 80)[82]
- ii) ligands that interact with receptors on the BBB to encourage RMT, e.g. transferrin peptide[83]
- iii) ligands that increase lipophilicity and decrease negative charge to encourage AMT, e.g. CLFFA[84]
- iv) ligands that prolong circulation time to increase exposure to uptake sites along the BBB, e.g. polyethylene glycol (PEG)[85].

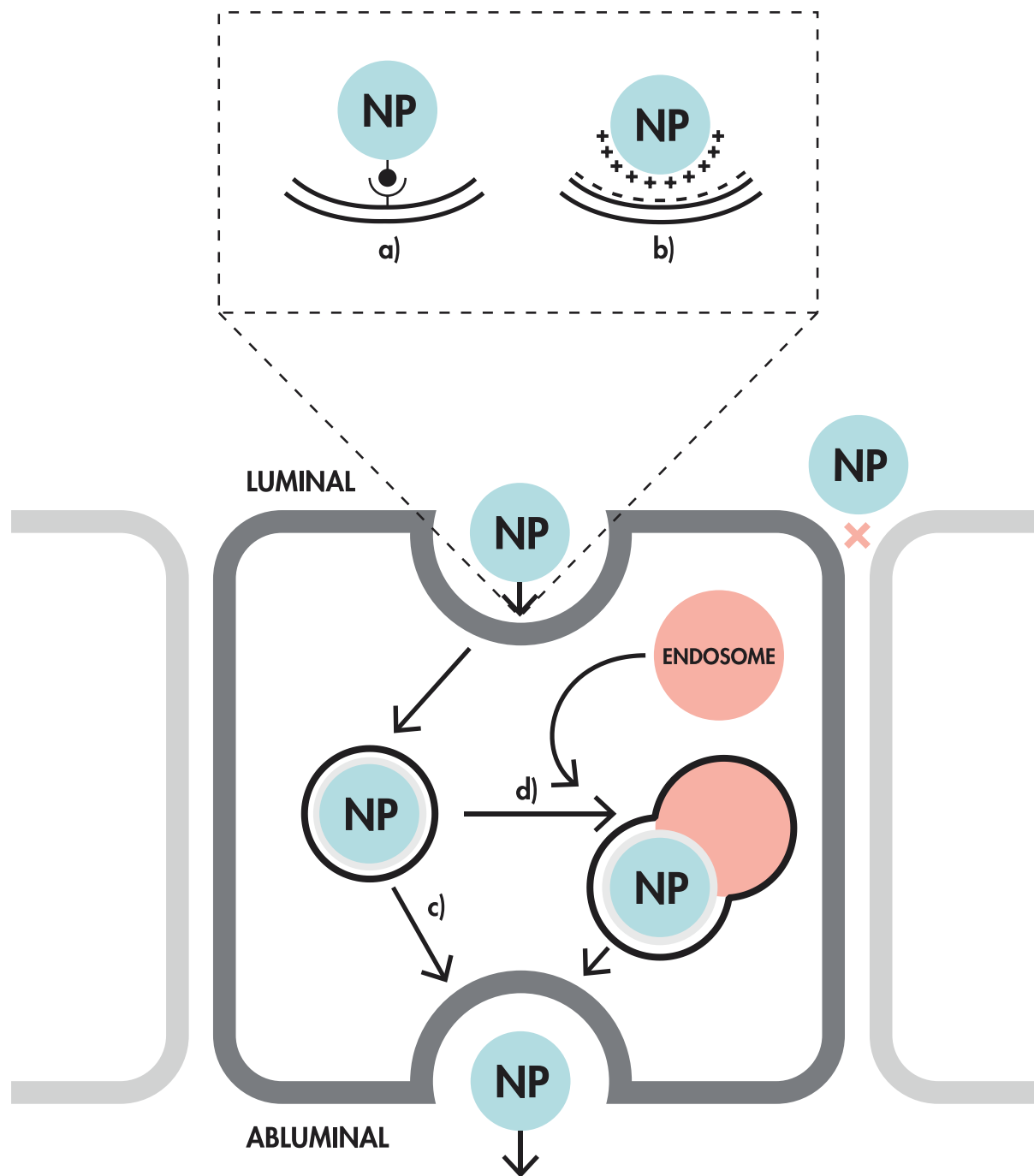
All these options can be applied to REE NPs.

### 5.2 Targeting glioblastoma uptake

It is possible that REEs may have a natural tendency to accumulate in astrocytic cancer cells, including GBM. This is because Zhuang *et al.*[86] found statistically significant higher concentrations of the REEs La, Ce, Gd, Lu and Sc in astrocytoma cells from human samples compared to age-matched autopsy controls. A possible explanation for this specific accumulation is that astrocytes are responsible for metal deposition and transport within the CNS, and their dysfunction in cancer leads to aberrant REE processing.[87] This does assume that cancer cells such as those in the GBM cells possess some similar physiological properties to that of normal astrocyte cells, an assumption inferred by the astrocyte model of GBM. It has even been proposed that astrocytes may even possess the capacity to accumulate REEs to less detriment compared to surrounding neurons.[88] This is due to the fact that astrocytes exhibit inherent transcriptional and expressional regulations of brain-derived neurotrophic factor (BDNF), which protects from oxidative stress[89] and govern  $\text{Ca}^{2+}$  and free ROS metabolism[90]; aspects associated with free REE exposure.



NPs have been functionalized with a number of targeting molecules to improve astrocyte uptake in animal models, including chlorotoxin (CTX)[91], anti-epidermal growth factor receptor (EGFR) antibody[92], and anti-transferrin receptor antibody[93]. Additionally, functionalizations have also been successfully shown to augment targeting of and internalization of NPs into GBM cells specifically. These include peptides such as the Arg-Gly-Asp (RGD) sequence and interleukin-13[94], iron-mimics targeting transferrin and transferrin receptor[95], and neurofilamental analogues of tubulin-binding sites[96]. Hence, it should be perfectly feasible to adapt the use of these molecules to combine the unique properties of REE nanoparticles with such targeting moities for better localization. For more detail regarding the interaction between NPs and functionalization in determining cellular fate within the brain interstitium, an excellent review by Song et al [97] describes how surface chemistry governs cellular tropism of NPs in the brain and in GBM – **STAR THIS**



**Figure 2:** A cross-section of an endothelial cell of the blood brain barrier (BBB) which demonstrates the transport of a nanoparticle (NP) from the luminal to abluminal surface. The NP must attach to the membrane from the luminal surface by either (a) receptor-mediated transcytosis (RMT) or (b) adsorptive-mediated transcytosis (AMT). Then transport through the BBB occurs via vesicles that derive from either (c) caveolae or (d) clathrin-coated pits to reach the abluminal side for exocytosis onto the abluminal surface. The NPs remain too large to cross the BBB between endothelial cells.

## 6. Rare earth nanoparticle delivery to glioblastoma

The two main delivery modalities for REE NPs to target GBM are discussed below, with neither proven superior to the other as of yet. However, the systemic exposure through intravenous delivery may modestly favor trialling direct intratumoral delivery at this point in time.

### *6.1 Intravenous delivery*

Administration via the circulation is the most widely accessible option in clinic for therapeutic drug delivery to GBM. However, the NPs are subject to a number of physiological obstacles including first-pass metabolism, circulation to all organs of the body and the need to efficiently overcome the BBB. One study investigating the intravenous delivery of naked NPs to stereotactically implanted GBM in one lateral ventricle of rats, made three significant conclusions regarding the intravenous approach [98]. Firstly, the greatest delivery efficiency of the NP to the brain tissue was 0.93% one hour after administration, whereas median delivery of NPs to a solid tumour organ is 0.7%.[99] Secondly, the concentration of NP formulation was greater in GBM tissue compared to healthy contralateral brain tissue. Thirdly, it was observed that six days after GBM implantation that the magnitude of BBB disruption was sufficient to permit entry of the NPs, presumably due to the EPR effect. It is a timely reminder that while the intravenous approach is able to take advantage of the natural BBB breakdown in the presence of GBM, the BBB is still present and needs to be effectively overcome, most likely by thorough NP design and optimisation.

### *6.2 Intratumoral delivery*

Delivery directly into the tumour bypasses the issues of the BBB by surgical intervention in a set-up similar to brain needle biopsy.[100] A recent study by Cohen et al., [101] analyzed the

effectiveness of intratumoral administration of a chemotherapy agent coupled to NPs into GBM in mice. Significantly increased survival in the experimental arm was observed compared to the control arm of mock-treated mice. These results imply a modest degree of success for this delivery approach. A future consideration in refining this approach is the circulation of the cerebral interstitial fluid which affects therapeutic movement intracranially.[102] This may influence the micro-distribution of NPs at delivery prior to their cellular uptake which poses a clinical dilemma because cellular perturbation by NPs to the surrounding non-cancerous cells in the GBM microenvironment has the potential to influence tumour biology.[103] This is exemplified by the success of the FDA-approved 1,3-bis-(2-chlor-oethyl)-1-nitrosurea (BCNU, Gliadel ®) wafer, a chemotherapy that is implanted directly into the tumor cavity after surgery. Initially, this approach was seen as a breakthrough as it provided direct targeting to a GBM site overcoming the physiological barriers facing intravenous delivery with modest clinical gains.[104] The issue today however, is that the pharmacokinetics of the drug still remain poorly defined in both the tumour and surrounding tissue.[105] There is no guarantee as of yet that NPs delivered intratumorally will not also suffer a similar fate.

## *7 Limitations for the use of REE NPs in GBM treatment*

### *7.11 Adverse effects in the brain*

If the EPR effect does not operate effectively, then REE NPs could permeate through brain tissue without specific directive to the GBM site, and deposit in surrounding healthy brain tissue. Zhao et al.[106] demonstrated *in vivo* mice that some REEs, such as Ce and Nd, have the ability to induce neuronal inflammation and edema in healthy brain tissue at a cellular level. These effects are associated with the potential anticancer cytotoxic mechanisms of REEs, and in the case of non-GBM neuronal death, includes lipid peroxidation, ROS production and downregulation of antioxidant activity.[29] Although such undesirable effects at a cellular level in healthy cells occur only at high concentrations of REEs, it remains uncertain as to what these concentrations are and their predictability. Furthermore, permeability, migration patterns, and clearance rates through healthy brain and tumor interstitium, as

well as affinity for particular neuronal cell types, and toxic threshold of REEs in the healthy brain and GBM are not clearly defined. While these concerns may be more relevant to intravenous delivery, in which all brain areas serviced by vasculature would be affected, intratumoral delivery could still expose healthy brain tissue to REEs albeit at a more localized level. Regardless, the possible induction of healthy neuronal death is a cause for concern, and demands thorough investigation in vitro and in vivo before translational studies commence.

It is currently unknown what the long-lasting effects of accumulated REE NPs at a GBM site in the brain may be; they may be detrimental to surrounding tissue, they may be cleared by the vascular and lymphatic systems, or they may simply be benign in-situ. If the NPs cannot be cleared, and pose a risk to non-cancerous tissue once their anticancer properties have been utilized, then minimally invasive extraction from the site may be a feasible option if they were delivered directly to the tumor or the resection cavity. Clearly, many surgical factors such as tumor or cavity size, anatomical location, NP delivery media will need to be considered.

#### *72 Adverse effects in the rest of the body*

As inorganic NPs, REE NPs may be sequestered from circulation and have the potential to accumulate at high levels in organs such as the kidneys and the liver. [52] **STAR THIS**. This is because the two primary excretion routes from systemic circulation for REE NPs are (i) the renal pathway as urine and (ii) the hepatic pathway as bile in faeces. Renal clearance occurs via glomerular filtration, can be a rapid process with minimal catabolism making it an ideal pathway for NPs to be cleared. However, it is difficult to predict how well unspecific REE NPs will pass through this pathway. This is due to the fact that although the glomerular fenestrations are physically 43 nm in diameter[107], physiologically they are significantly smaller at approximately 5 nm in diameter due to the summative effects of the adjacent glomerular capillary layers[108]. For NPs that cannot pass through the glomerular fenestrations, they enter the hepatic pathway for clearance. The liver is the primary site for

catabolization and elimination of foreign materials into the biliary system. It has been reported that the liver most efficiently eliminates NPs up to the size of 20 nm.[109]

There is a concern of local toxicity if NPs do not clear from the body and accumulate in excretory organs. If REE NPs were to accumulate in the kidney, REEs such as La, Ce, and Nd could be extremely nephrotoxic at high doses, capable of inducing nephritis, epithelial cell necrosis and oxidative stress to kidney which ultimately may lead to organ failure.[110] Of all organs in the body however, there is a distinct predominance for inorganic NPs up to 250 nm in size to rapidly accumulate in the liver, with only slow clearance the alleviating process.[111] This poses a unique situation where the risk of REE NP breakdown in the catabolic liver can affect liver function. At high doses, REEs such as Ce, Pr and Gd can induce many unwanted hepatotoxic effects, such as hepatitis, steatosis, jaundice, hematological derangement and necrosis.[112, 113] Interestingly, REEs may also be cytoprotective in the liver, as evidence exists of mitogenesis promotion in response to insult and P450-dependent monooxygenase inhibition.[114, 115] Nevertheless, determining the threshold for nephrotoxic and hepatotoxic effects is a significant goal regarding the use of REE in NP formulation targeting the brain. Approaches that will complement this include designing ultra-small REE NPs to favor renal excretion and functionalizing NP surface chemistry to resist hepatic catabolism and encourage greater clearance.

## **8. Conclusion**

The prognosis of glioblastoma has remained dismal for many years and there is a need to investigate novel therapeutic options. The use of rare earth elements nanoparticles is a novel concept, however there is a significant theoretical base to justify investigation. These elements can be used to target and affect glioblastoma cells both in the presence or absence of radiation therapy. In a nanoparticle formulation, they exist as an effective means of delivery to the glioblastoma at a physiological level and can be incorporated into different administration options at a clinical level. There remain multiple translational unknowns regarding rare earth element nanoparticles and their threshold for toxic effect in the brain and body. Nonetheless, the concept of rare earth element nanoparticles ameliorating glioblastoma prognosis is a relatively novel, but exciting, area of research at the intersection of medicine, science and engineering.

## **9. Future perspective**

The future of incorporating rare earth elements into nanoparticles for optimising glioblastoma treatment is conceivable. Clinically, in a similar notion to how today rare earth gadolinium contrast agents are delivered intravenously for brain tumours diagnosis by magnetic resonance imaging, it is not unimaginable that one day nanoparticle formulations based on rare earth elements may be delivered similarly to elicit therapy for these tumours as well. Indeed, this is at the core of the emerging field of theranostics. Furthermore, intratumoural delivery after surgical resection of glioblastoma is pragmatic and reasonable in vivo, as procedurally it is more appending than substituting to current protocol.

The current protocol for treating glioblastoma after resection is radiation therapy and chemotherapy. Based on current trajectories, this will not change in the near future. However, the ability to augment components of the protocol by readily-accessible and -produced rare earth nanoparticle formulations is realizable. Thus more plausible in concept for translational Phase I clinical trials than more radical novel considerations we have to date once the safety profile of this potential therapy can be established.

## EXECUTIVE SUMMARY

### *Glioblastoma*

- Glioblastoma (GBM) have been treated by surgery, followed by radiation therapy and chemotherapy for the last decade without improvement to mean survival at diagnosis of one year.
- It is most studied using an astrocyte model, which assumes astrocyte cells share cytological similarities with glioblastoma cells.

### *Rare earth element nanoparticles in the brain*

- Rare earth elements (REE) are readily found in nature and in humans at non-toxic levels.
- REE have unique electronic configuration involving 4f orbitals.
- These commonly exist as oxides, which can be readily formulated in nanoparticles (NPs)
- REE NPs have successfully been used as contrast agents.

### Mechanisms of REE NPs against GBM

- REE ionic radii are similar to that of calcium, a key regulator of cellular function, and can in certain circumstances then disrupt vital cellular processes to induce a cytotoxicity.
- REE electronic configuration is able to facilitate electron ejection by the Auger effect when exposed to radiation, which leads to increased generation of toxic radical oxygen species to augment radiation effect.
- Their energy-level pattern can produce a luminescence by upconversion upon exposure to near infrared light to stimulate associated photosensitising cytotoxic chemotherapy agents.

### *Designing REE NPs for GBM*



- Size is the principal parameter in design as it influences NP penetration of the blood brain barrier, retention in the body, and GBM cell uptake.
- Shape influences the probability and speed of nanoparticle uptake into GBM cell.
- Surface charge influences the interaction of the NP with the cell membrane, with electroattractive positive charges more favoured for cancer cell uptake.

#### *Reaching GBM with REE NPs*

- NPs are small enough to penetrate through the natural blood brain barrier (BBB) via either receptor-mediated or adsorptive-mediated transcytosis to reach GBM.
- In the presence of glioblastoma, the integrity of the BBB deteriorates due to the enhanced permeability and retention effect which enhances NP delivery to glioblastoma.
- NPs can also be functionalised by a number of different ligands to enhance blood brain barrier penetration and GBM cell uptake.

#### *REE NP delivery options to GBM and limitations*

- Optimal delivery mode is still unknown as both intravenous delivery and intratumoral delivery both have advantages and uncertainties.
- REE NPs have neurotoxic potential within the brain.
- REE NPs have nephrotoxic and hepatotoxic potentials within the body.

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