1.1.Brain Tumor

Brain tumors refer to a heterogeneous group of primary and metastatic neoplasms in the central nervous system and are one of the life-threatening diseases which are characterized by low survival rate [1]. According to a worldwide survey, approximately 24,000 cases of primary malignant brain tumors have been projected annually [1, 2]. According to American Brain Tumor Association (ABTA) statistics, nearly 80,000 new cases of primary brain tumors are expected to be diagnosed in 2017 worldwide [2]. In India the prevalence of brain tumor is around 2.8 per 1 lakh persons. The estimated incidence, mortality and 5 year prevalence of brain tumor among men in India is approximately 11855, 9574 and 17251 respectively which represents 2.5%, 2.7% and 2.6% of Indian population respectively while for women it is 6976 (1.3%), 5578 (1.7%) and 10157 (1%) respectively. Depending on the origin of the tumor, they are grouped as tumors of neuroepithelial tissue, tumors of cranial and spinal nerve, tumors of meninges, heamopoitic origin neoplasm and lymphomas, tumor of sellar region, germ cell tumors and cysts [5]**.** Though there are many types, tumors of epithelial lining of neurons (glioblastomas and astrocytomas) have the highest rate of occurrence followed by the tumors of the meninges, tumors of nerves and tumors of sellar region [6, 7]. Most patients with brain tumors eventually succumb to the disease despite aggressive treatment approaches. The median overall survival of patients having brain cancer is only about three years for anaplastic astrocytomas and around 14.6 months for GBM (glioblastomas) patients after current multimodal treatment-aggressive surgical resection followed by concurrent or sequential therapy [8, 9]. Brain metastases are another important class of tumors in the central nervous system originating mainly from systemic cancers in the lung, breast and skin [10]. Glioma is the most frequent primary brain cancer which accounts for 29% of all primary brain and CNS tumors and 80% of malignant brain tumors. These malignant gliomas are primary tumors that are derived from glial origin and account for approximately 70% of new primary brain cancer diagnosis [11, 12]. WHO has classified glioma in three category viz. astrocytoma, oligodendrogliomas and mixed gliomas (oligoastrocyotomas). Amongst gliomas, glioblastomamultiforme (GBM) which is a grade IV astrocytoma according to the World Health Organization (WHO) classification, is the most common and aggressive form of glioma in nature [13]. The high mortality rate due to GBM can be attributed to specific properties of glioma which includes highly infiltrative nature and lack of clear margin. The existing therapy for GBM is nonspecific and almost fails to prevent reoccurrence of disease.

1.2. Current treatment approaches for brain tumor

Multiple treatment approaches have been experimented for treatment of brain cancer. The multidisciplinary approach for treatment of brain tumor includes combination of surgery, radiation and chemotherapy. Chemotherapy is the most common initial therapy for brain cancers. Temozolomide is the first generation agent used for the treatment of brain tumors and is presently given orally [21]. Other agents indicated for being used in treatment of brain cancer include irinotecan, carmustine, cisplatin and lomustine. Most of the drugs have shown enhanced anticancer activity for brain cancers *in vitro*. However, clinical failure was observed with such drugs due to insufficient barrier passage. Currently employed chemotherapeutic agents for treatment of gliomas with few of the relevant physicochemical properties are enlisted in Table 1.1 [22]. Although chemotherapy in combination with other treatment approaches such as radiotherapy and surgery has proved effective still tumor recurrence (96% cases) adjacent to resection margin after surgical resection makes it important to develop adjuvant therapy which can help minimize the recurrence with enhanced efficacy and specificity. Gene therapy and toxin therapy have been proposed for treatment of brain cancers [23, 24]. Although most of these therapies have not been commercialized, few of the products are in clinical trials and may soon make way to the commercial market. Clinical trials of gene therapy for brain tumors to date have been focused on phase I toxicity evaluations. None of them have shown significant efficacy and many point to high sensitivity of normal brain and related consequences. Immunotoxins were first shown to be potent cancel cell killers in 1970s after which lot of research have been conducted for exploring them for treatment of brain cancers [25-27]. Clinical and promising preclinical studies in brain tumor have been summarized in Table 1.2.

Table 1.1: Chemotherapeutic agents with their physicochemical properties for treatment of gliomas

Agent	**Mechanism**	**Permea-bility coefficient**	**Brain tumor**	**Mol. mass**	**Half life**	**LogP**	**BBB passage**
Carmustine	Alkylating agent	$0.92*10^{-4}$ $cm.^{s-1}$	GBM	214.04	15-30 min	1.5	Yes
Lomustine	Alkylating agent	$3.16*10^{-4}$ $cm.s^{-1}$	Oligodendrogliomas	233.69	94 min	3	-
Methotrexate	Inhibition of DHFR	$1.77*10^{-7}$ $cm.s^{-1}$	CNS Lymphomas	454.44	3-15 hrs	-0.91	
Imatinib mesylate	Inhibition of tyrosine kinase enzyme	-	GBM, CNS lymphomas	493.60	18 hrs, Metabolite 40hrs	1.198	-
Temozolamide	Alkylating agent	-	GBM, Astroctomas, Oligodendrogliomas	194.15	1.8hrs	-0.99	Yes
Vincristine	Inhibition of mitosis at metaphase through its interaction with tubulin	$1.58*10^{-7}$ $cm.s^{-1}$	oligodendrogliomas	923.04	19-155 hrs	2.8	No
Procarbazine	Breaking of DNA Strands	$3.01*10^{-5}$ $cm.s^{-1}$	GBM, Astroctomas, Oligodendrogliomas	221.22	10 mins	0.06	Yes
Cisplatin	Crosslinking of DNA	-	GBM	300.05	40-45 mins	-	-
Cyclophosphamide	Alkylating agents	-	GBM	261.08	3-12 hours	0.8	No
Everolimus	mTOR inhibitors, selective immunosuppressants	-	GBM, Astroctomas, Oligodendrogliomas	958.22	~30 hours	5.01	No

Table 1.2: Targeted toxins used against brain tumors

Immunotoxin	Clinical phase	Status	Outcomes	Adverse effect	Ref.
D2C7(scdsFv)-PE38 (D2C7-IT)	I/II	Ongoing	N/A	Headache, seizure, weakness, dysphasia	28
IL-4(38-37)-PE38KDEL (cpIL4-PE)	I/II	Ongoing	Median survival: 4.7 months; six-month survival: 36%. Median survival : 42.7 weeks in phase II and 36.4 weeks in phase III	Headache, dysphasia, seizure, weakness, pulmonary embolism	29
IL13-PE38QQR (IL-13PE)	I/II/III	Not Active	Median survival 28 weeks (95% CI, 4.1–45.1)	Hemiparesis, fatigue, headache,	30
TGFα-PE38 (TP38)	I	Discontinued	Median survival 37 weeks (95% CI, 26–49);	NA	31

1.3.1. Limitations to current chemotherapy

Brain allows passage of some of endogenous material, a few hydrophobic agents and particles with molecular weight of less than 500Da. Hence, lipophilicity of the drug is one of the important factors which should be considered while designing new entities for treatment of tumors [32]. The decrease in efficiency of chemotherapy is due to enhanced efflux which might lead to poor tissue retention and short biological action (Figure 1.1). Due to this the benefits of chemotherapy are controversial, with clinical trials showing wide variation in whether or not, and to what degree, chemotherapy confers a survival benefit in these patients [33, 34]. Another pre-requisite for enhanced efficiency of chemotherapy is penetration or accumulation of drug inside tumor which is hampered owing to various barriers among which Blood Brain Barrier (BBB) plays the most important role [35].

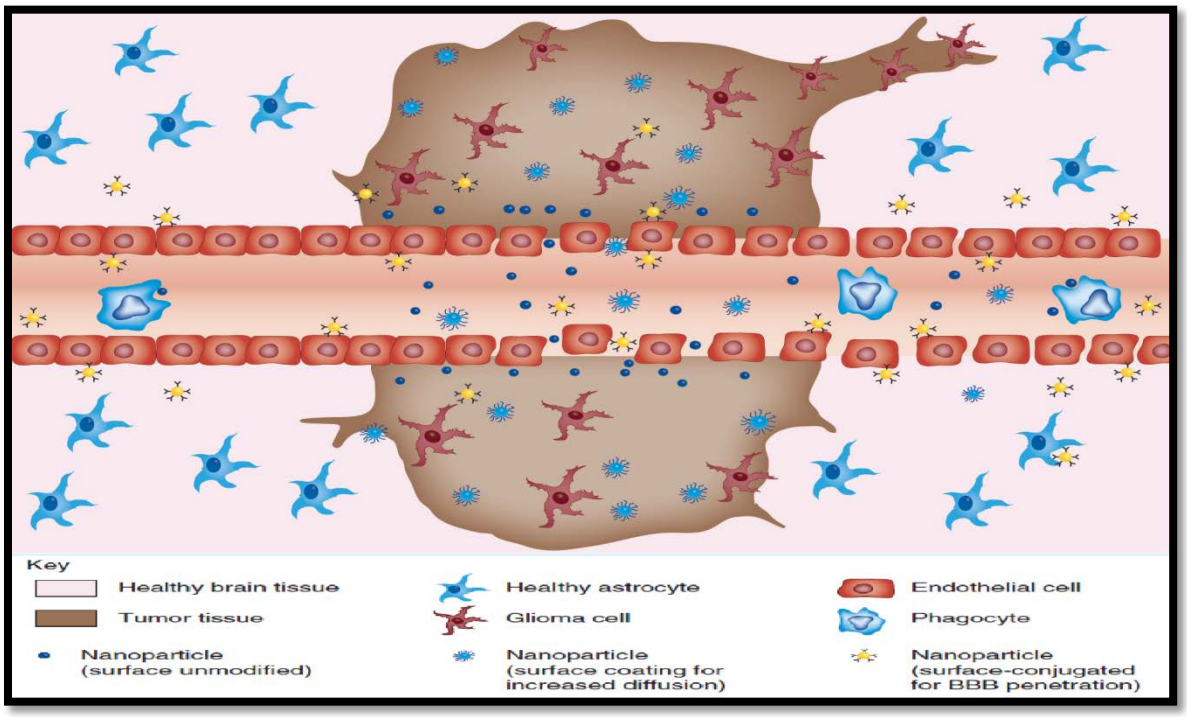

Figure 1.1: Major barriers in nanoparticle delivery to malignant glioma. These barriers pose potential limitation for nanoparticle delivery to brain tumor and can be overcome by various particle modifications, such as 'stealth' surface coating, exploitation of the enhanced permeation and retention effect, or conjugation with BBB-penetrating or –binding molecules. **(A)** Clearance by immune cells; short half-life, **(B)** BBB, **(C)** insufficient diffusion through tissue, **(D)** non-specific delivery to healthy cells and **(E)** tumor invasion. BBB: Blood–brain barrier.

BBB protects neurons from systemically circulating potentially cytotoxic agents. Cerebral micro vessels are lined by the endothelial cells covering an array of passive and active features forming a selective barrier allowing the passage of only selective material [36, 37]. Specific transporters present on the luminal and abluminal surface of the BBB endothelial membranes regulate passage of small hydrophilic molecules, providing a selective "transport barrier". This protective mechanism permits the entry of required nutrients along with efflux of potentially harmful compounds. Transport of hydrophilic molecules, if occurred is either by specific receptor mediated transcytosis or by less specific adsorptive mediated transcytosis [38]. Hence, the BBB prevents the influx of harmful endogenous and exogenous molecules from the bloodstream but also becomes a major limiting factor for anti-brain tumor therapy. Additionally, ATP-binding cassette transporters such as P-glycoprotein act as drug efflux transporters and their high expression limits substrate transportation across the BBB. Along the Blood Brain Barrier the efflux transporters (Figure 1.2) like P-gp, MRP‟s like MRP1, MRP2, MRP4, MRP5, GLUT1, Oatp2, Oatp3, ABCG2 etc. are expressed on the luminal side of the barrier offering resistance to uptake of active components [39-41].

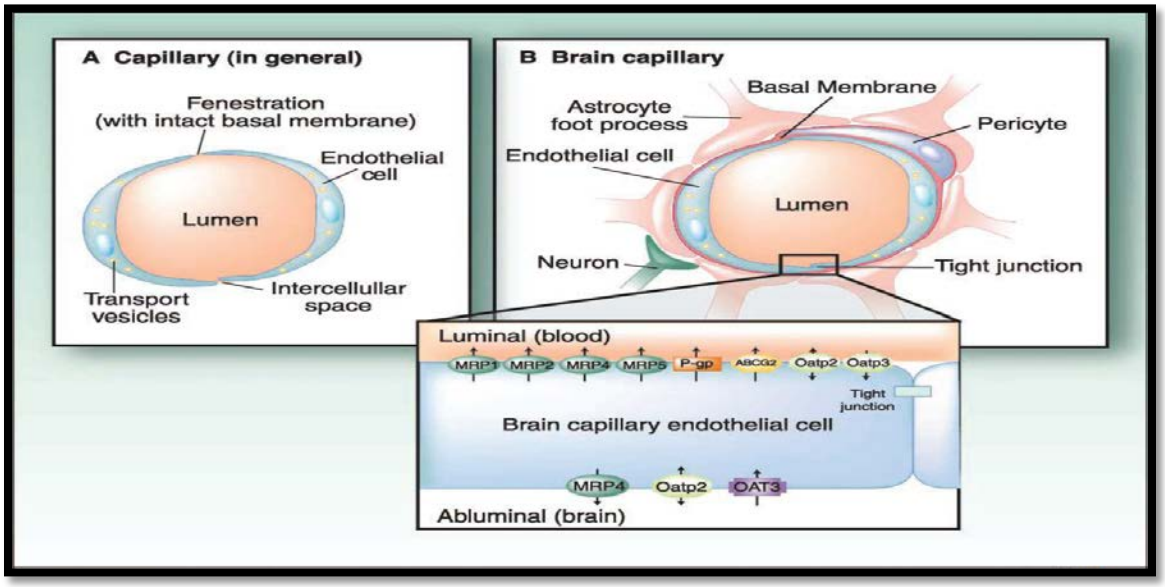

Figure 1.2: Efflux transporters expressed on BBB.

Blood–cerebrospinal fluid barrier (CSF) is the second barrier that blocks the passage of systemically administered therapeutic agents [42]. It is formed by tightly bound choroid epithelial cells, which regulate molecule penetration within the interstitial fluid of the brain parenchyma. This barrier prevents most macromolecules from passing into the CSF through the

bloodstream [43]. Organic anion transporter 3 (Oat 3), peptide transporter 2 (PEPT2), and P-glycoproteins (P-gp) acts as an outward efflux systems, and these pumps are able to decrease the CSF concentration of several antibiotics (e.g. penicillins), chemotherapeutic agents (e.g. methotrexate) [44, 45], and HIV proteinase inhibitors (e.g. ritonavir and atazanavir) [46, 47].

The blood–tumor barrier in the tumor forms a third barrier for transporting therapeutic agents [48, 49].Unlike normal brain capillaries; the tight junctions of endothelial cells in the tumor are significantly compromised. The high Intratumoral interstitial pressure created by the leaky tumor vasculature limits the penetration of drug from the bloodstream into the tumor [50].

1.4. Nanoformulations for treatment of brain cancer

Selective targeting of cancerous cells has always been a matter of concern while developing carriers for delivering anticancer agents. Although chemotherapy has been most widely used modality for treatment of cancer for several decades, it suffers from various limitations such as non selective distribution of drugs, toxicity to normal cells, undesirable side effects and development of resistance [51-53]. These limitations demand development of delivery systems which can specifically target drug to tumor cells along with controlled release and targeting of drug for enhancing intracellular localization which will ultimately lead to decrease in non specific cell toxicity and side effects [54, 55]. Nanoparticle based drug delivery systems have opened new avenues for treatment of gliomas and other types of brain tumors. Nanocarriers can be applied for medical treatment, imaging, diagnosis, drug delivery and tissue regeneration due to their promising nature [56]. Due to the feature of modifiability possessed by the nanoparticles, they reduce the peripheral side – effects and increase the relative amounts of drug reaching the brain by crossing the barrier. In the recent past, these nanoparticles based formulation for targeting brain tumors have gained wide attention leading to extensive research in this area. Various nano-formulations for treatment of brain tumor which are under clinical trials have been summarized in Table 1.3. Owing to their small size, these nano-formulations confer better permeability of therapeutic agents into cells compared to conventional therapy. Particles having size less than 200 nm can easily pass the blood tumor barrier due to leaky vasculature and around 100 nm size surface modified particles can circumvent the barrier. A large number of compounds including antibodies, proteins, peptides, chemotherapeutic agents have been delivered using nanoparticles [57-65].

Table 1.3: Various nano-formulations for treatment of brain tumor which are under clinical trials

Material	Particle type	Structure formed	Size (nm)	Component	Application	Development Phase	Ref.
Organic	Liposome	Colloidal vesicular structure	10-1000	Polymer	Drug carrier	Marketed	66
	Micelles	Nanosphere, cylinder	20-200	Polymer	Drug carrier	Preclinical	67
	Polymeric nanoparticle	Nanosphere, Nanocapsules	10-1000	Polymer	Drug carrier	Preclinical	68
	Dendrimers	Branch	>5	Poly(amidoamine)	Drug carrier	Preclinical	69
Inorganic	Gold Nanoparticles	Nanospheres; Nanorods; Nanoshells	1-100	Gold	Drug carrier, Photothermal therapy	Preclinical	70
	Iron oxide nanoparticles	Nanospheres	50	Iron oxide	Drug carrier, Hyperthermia, MRI	Phase I, II	71
	Ferromagnetic disks	Microdisk	1000	Iron-Nickel	Magneto-mechanical stimulation	Preclinical	72
	Ceramic nanoparticles	Nanospheres	20-100	Silica	Drug carrier	Preclinical	73
	Quantum dots	Nanorods, Nanospheres	20	Cadmium, Selenide	Fluorescence Imaging,	Preclinical	74
	Titanium dioxide	Spheres	5	Titanium	Photodynamic therapy	Preclinical	75

Nanoparticles due to small size and modifiability warrant their selective uptake by tumor cells. They can be formulated out of variety of substances and can carry multiple loads of drugs directing the substance towards the tumor cells. Due to the feature of modifiability possessed by the nanoparticles, they reduce the peripheral side – effects and increase the relative amounts of drug reaching the brain by crossing the barrier [76-78]. The key features of nanoparticles which make them suitable drug carrier for targeting brain cancer includes composition, unique physical properties, passive targeting abilities, as well as tunable surface functionality for active targeting (Figure 1.3).

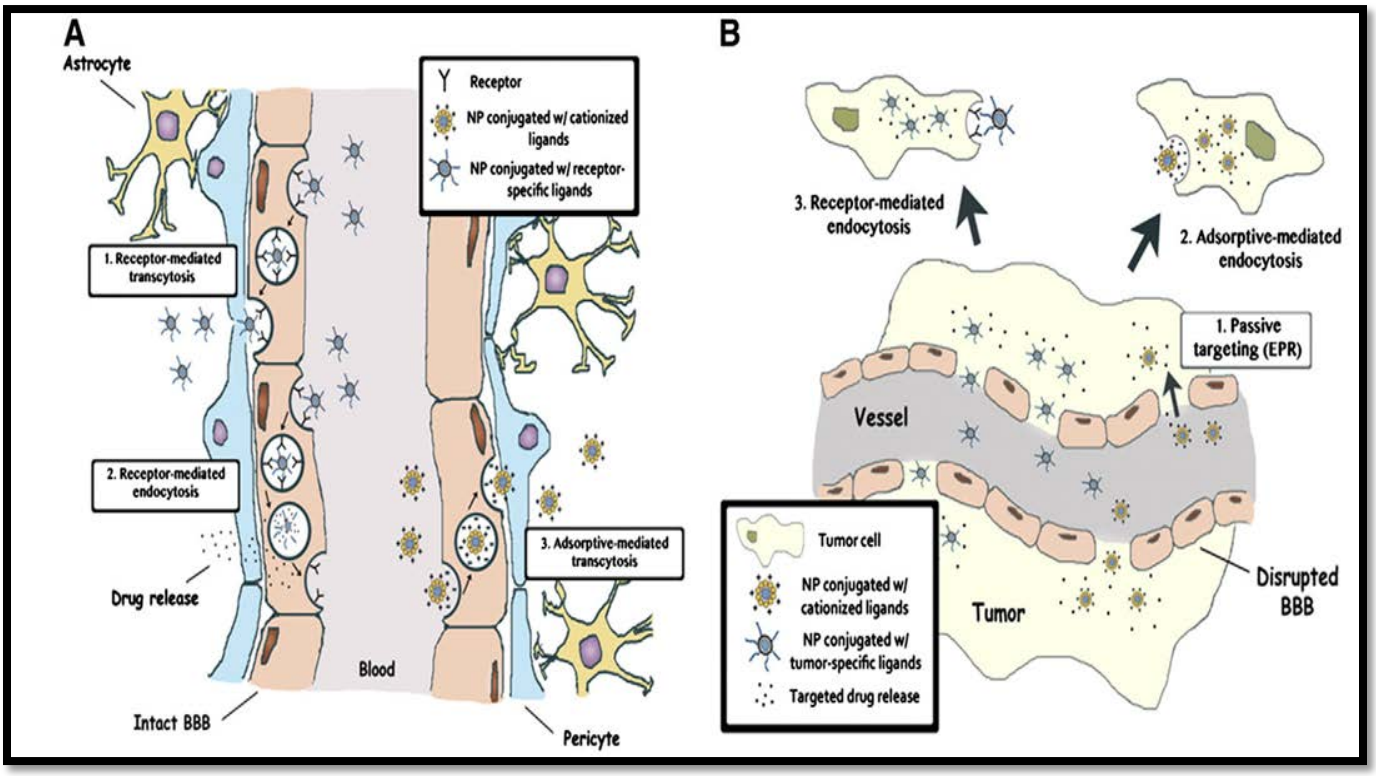

Figure 1.3**:** Transportation mechanisms of multifunctional nanoparticles into the brain tumor. (A) Transport of multifunctional nanoparticles across the BBB: 1) receptor-mediated transcytosis, 2) receptor-mediated endocytosis, 3) adsorptive-mediated transcytosis of nanoparticles with cationized ligands. (B) Mechanisms of transportation across the disrupted BBB and selective targeting of brain tumor cells: 1) passive targeting via the EPR effect, 2) adsorptive-mediated endocytosis or 3) receptor-mediated endocytosis. Both mechanisms offer a targeted delivery to brain cancer cells, sparing the normal tissue. NP: nanoparticle.

A variety of nanoparticles with well-defined shapes such as solid spheres, rods, tubes, and other complex shapes can be designed and synthesized by top down or bottom up techniques. Nanoparticles have large surface to volume ratios that contribute to their high loading capacity. As drug delivery systems, nanoparticles have been shown to improve drug solubility, prolong blood circulation half-life, and control drug-release [79-81]. Furthermore, the payload can be extended to include imaging probes and contrast agents. For example, organic fluorescence probes can be incorporated into the nanoparticle structure for particle tracking [82, 83].

1.5. Targeting brain tumors

In recent years, plenty of strategies have been developed for overcoming the BBB and/or targeting to glioma such as receptor, transporter, or adsorption-mediated drug delivery according to different transport mechanisms [84, 85]. Both passive targeting and active targeting have been explored for targeting brain tumor. Performance of particles designed for passive targeting can be modulated by adsorption of solubiliser/ stabiliser like Polysorbate 80, Poloxamer of various grades **(**Polyethylene Glycols and Poloxamines [86-91]. These polymers not only prevent RES uptake but also prevent endosomal uptake of the particles. This is of significance for particles intended for long circulation and targeting to a particular organs. Alternatively, transport may be realized by transcytosis of the NPs loaded with drug across the endothelial cells.

Another strategy under investigation avoids damage to the non cancerous cells by chemotherapeutic agents. This strategy exploits and utilizes the over expressed receptors on the tumor cells. A specific marker/ target/ receptor is expressed on disease associated cells at a much higher level than on normal cells. Agents that can potentially target these receptors are attached to the carrier system enabling the cargo to specifically deliver the drug to cancerous cells due to binding of Ligand to the over expressed receptors [92-94]. Various ligands for targeting BBB/glioma different receptors including transferrin receptor (TfR), lactoferrin receptor (LfR), low-density lipoprotein receptor (LDLR), and folate receptor (FR) is summarized in figure 1.4 [95]. Antibodies, transferrin, insulin, apoliprotein, ferritin, macroglobulins, Amyloid precursor proteins, Plasminogen Activator Inhibitor coated / conjugated nanoparticles primarily use receptor mediated endocytosis mechanism [96]. The advantages of using such ligands are that it makes the material more substrate specific; i.e. it becomes more site specific and also reduces the toxicity/side effects to other cells due to their site specificity.

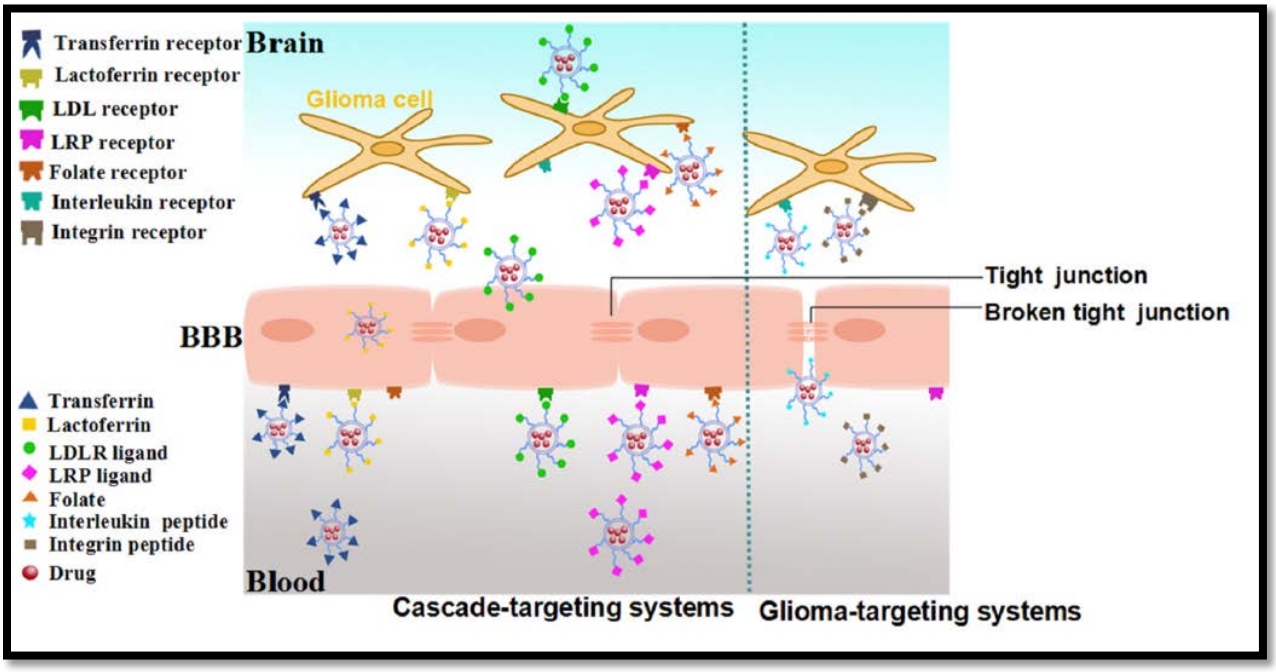

Figure 1.4: Representation of brain cancer targeted nanoparticles system

1.6. Simultaneous therapy and imaging for brain tumors (Theranostic Nanoparticles)

In the recent past, nanotechnology has garnered much attention due to its potential application in cancer, and the National Cancer Institute has constituted an Alliance of Nanotechnology in Cancer with focus on the development of novel nanoplatform- based diagnostics, therapeutics and preventive agents [97]. Nanoparticles are colloidal particles (10-100 nm in size) typically synthesized in either aqueous or organic phases. Due to their small size, nanoparticles can easily flow through blood capillaries and enter the target cancer cells [98, 99]. Encapsulation or conjugation of ligands which can provide imaging capabilities to nanoparticles will further monitor targeting of nanoparticles for tumor targeting. These objectives can be achieved by developing multifunctional carriers which can fulfill required criteria for development of tumor target delivery systems. Such nanocarrier based system for multimodal therapy and diagnosis of tumor provide both therapeutic and diagnostic ability to nanoparticles (Figure 1.5). The term theranostics encompasses two distinct definitions as defined by the combination of therapeutic and diagnostic agents on a single platform. This interesting approach aims to develop multimodal theranostics systems that use co-encapsulation of multiple different diagnostic modalities and therapeutic in targeting nanomedicine platforms [100]. As an example of studies published in

recent years, it highlights the combination of a therapeutic effect of a traditional chemotherapeutic drug (i.e. paclitaxel, doxorubicin) and excitable probing agent for imaging (i.e quantum dots, gold or metals). Radiotracer-based imaging either using single-photon emission computed tomography (SPECT) or positron-emission tomography (PET) is particularly suited for targeted *in vivo* molecular imaging [101, 102].

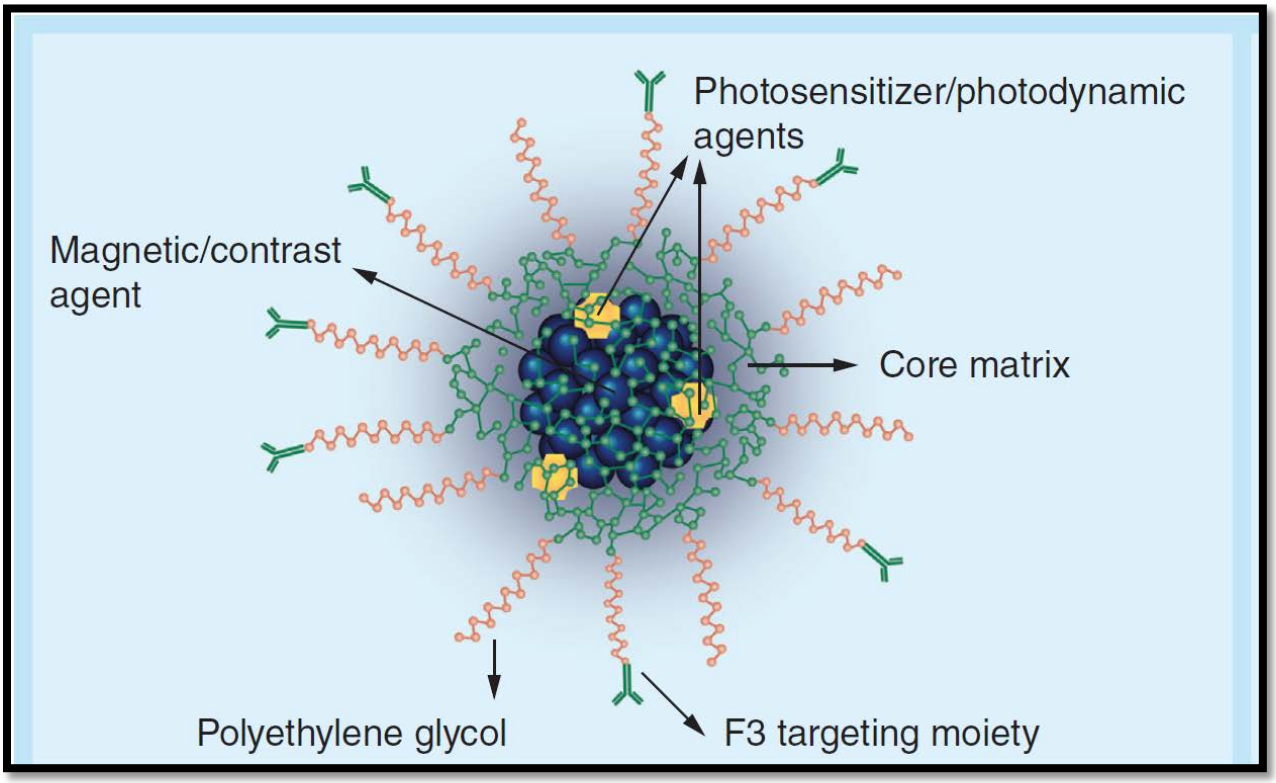

Figure 1.5: Schematic representation of theranostic nanoparticles

The major advantages of SPECT or PET molecular imaging techniques over other approaches include high sensitivity, the ability to make quantitative measurements, and the absence of a tissue penetration limit [103]. In addition, multimodality imaging (such as, PET/SPECT, MRI, CT, NIRF) can allow detecting the nanoparticle with various imaging techniques, providing more accurate and dependable data than SPECT or PET alone. These abilities make theranostic nanoparticles as the potential platform for effective multimodal treatment as well as imaging of tumors [104, 105]. The incorporation of multiple functions into a nanoparticle system would be highly beneficial for clinical translation. With the combination of imaging and carrying capabilities, nanoparticles could allow for the simultaneous delivery of

therapeutic agents to the tumor area and real-time tracking of their biodistribution and fate in vivo [106].

1.7. Intranasal Route For Targeting Brain Tumor

The abovementioned approaches will be beneficial only when the strategically developed nanoparticles are able to penetrate BBB and reach tumor site. In the past decade a number of drug delivery strategies have been developed to overcome challenges presented by the BBB. In particular, direct drug administration into the brain parenchyma, such as convection enhanced delivery (CED), has shown promising results in both animal models and clinical trials. CED is a continuous infusion that uses a convective (versus diffusive) flow to drive the therapeutic agent throughout a larger region of tissue [107-109]. This technique is well suited for the delivery of liposome and particulate drug carriers, which have the potential to provide a sustained level of drug and to reach cellular targets with improved specificity. However, CED requires the use of potentially risky surgical procedures to position the catheter into the patient's brain parenchyma. The convective flow to distribute the drug through the implanted catheter leads to measurable and significant inflammation and local edema because the drug solution infuses continuously beyond the tumor boundary into the adjacent normal brain tissue. This 'spillover' of drug to unwanted brain regions may be due to the pressure gradient of the convective bulk flow of CED and could lead to neural toxicity [110]. One technique that holds promise for bypassing the BBB to deliver drugs to the brain and eliminating the surgical risk and the spillover effect of drug to normal tissue is intranasal delivery. Intranasal delivery provides a practical, noninvasive method for delivering therapeutic agents to the brain because of the unique anatomic connections provided by the olfactory and trigeminal nerves (Figure 1.6). These nerves connect the nasal mucosa and central nervous system (CNS), allowing them to detect odors and other chemical stimuli. Intranasally administered drugs reach the brain parenchyma, spinal cord, and cerebrospinal fluid (CSF) within minutes by using an extracellular route through perineural and/or perivascular channels along the olfactory and trigeminal nerves without binding to any receptor or using axonal transport [111-114].

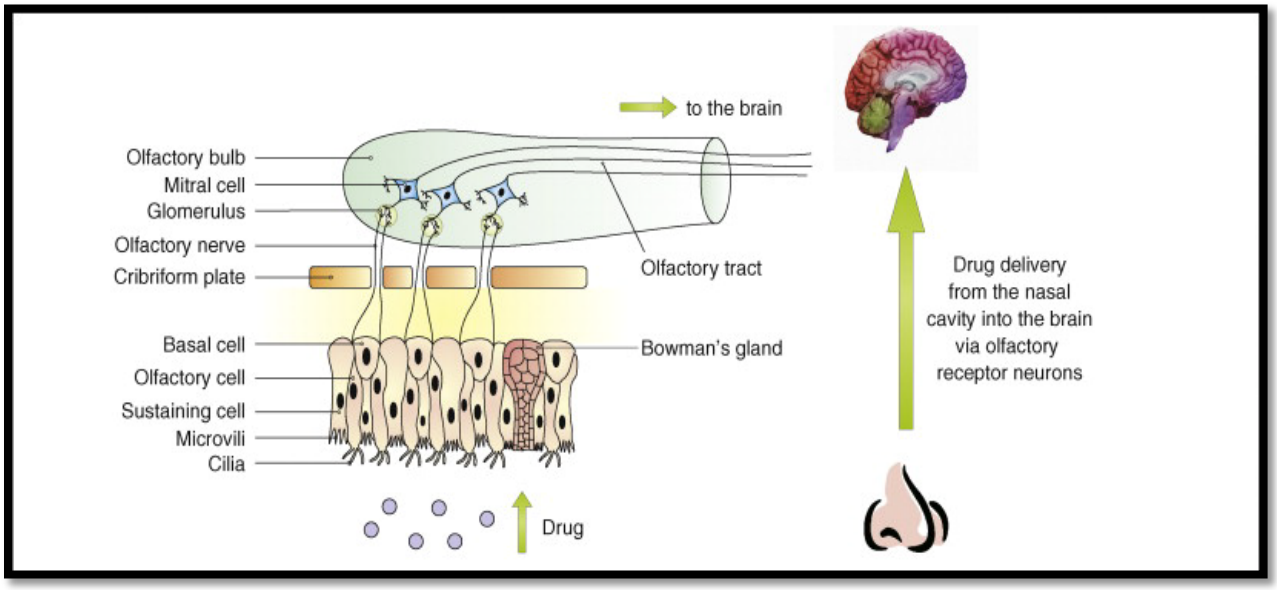

Figure 1.6: Nose to brain transfer of drug via. Olfactory pathway

In addition to bypassing the BBB, advantages of intranasal delivery include rapid delivery to the CNS, avoidance of hepatic first-pass drug metabolism, and elimination of the need for systemic delivery, thereby reducing unwanted systemic side effects. Intranasal delivery also provides painless and convenient self administration for patients, features that encourage its use for delivering therapeutic agents into the CNS. In brain tumors, anticancer agents such as methotrexate, 5-fluorouracil, and raltitrexed have been delivered successfully to the brain using intranasal delivery. Shingaki et al., reported that intranasally delivered methotrexate reaches the CSF and reduces tumor weight in rodent glioma allografts. Intranasal drug targeting to the brain of the chemotherapeutic raltitrexed is significantly higher than that with intravenous administration [115]. Recently, two different therapeutic agents, including a glioma-adapted vesicular stomatitis virus strain, VSVrp30a, and an oligonucleotide telomerase inhibitor, GRN163, have been used to selectively target malignant gliomas and have shown impressive oncolytic activity without harming normal brain tissue. Both studies utilized intranasal delivery, resulting in targeted and effective delivery of the therapeutic agents to the tumor and inhibition of the tumor growth in human GBM xenografts. In addition, intranasal delivery of the telomerase inhibitor GRN163 doubled the survival time for xenografted animals without apparent toxicity. Although intranasal GRN163 delivery is extracellular, as described above, intranasal VSVrp30a likely involves viral transmission within the olfactory neural pathway to the brain. These

findings support further development of intranasal VSVrp30a and GRN163 as potential therapies for brain tumor patients and perhaps as a means for treating multifocal brain tumors such as metastasis brain tumors and/or pediatric brainstem tumors, which are less amenable to potentially risky surgical procedures. Telomerase inhibitors, including GRN163, have reached the stage of clinical trials, so may soon become part of the available therapeutic armamentarium for cancer. Given the promising results from recent animal studies, intranasal therapeutic agents would seem to be prime candidates for clinical trials in patients with brain tumors. Initial trials of intranasal perillyl alcohol have begun in patients with recurrent malignant gliomas, and a reduction in the size of the brain tumors has been reported [116, 117].

1.8. Polyethylenimine

It has been shown that cationic polymers such as poly-arginine enhance nasal absorption of a hydrophilic model drug by acting on the tight junctional protein ZO-1 [118]. PEI also belongs to family of cationic polymers carrying positive surface charges that interact with the negatively charged cell membrane, are readily endocytosed by many cell types. It has been reported that similar to poly (arginine), PEI increases drug absorption by increasing paracellular permeability [119, 120]. *In vitro* study using liposomes made of phospahtidyl serine demonstrated that bPEI caused more destruction of liposomal membrane as compared to lPEI [121]. This suggests that PEI can destabilize or disrupt plasma membrane. In a study conducted to analyze stress and toxicity pathways triggered by PEI, it was observed that endocytic uptake of PEI caused swelling and rupture of endosomes causing intracellular stress and mitochondrial alterations, finally leading to apoptotic cell death at higher doses [122]. Previous research has shown that PEI is able to depolarize mitochondria leading to increased caspase-9 activity, decreased mitochondrial membrane potential, and increased phosphatidylserine exposure as early as one hour after treatments with PEI polyplexes at an N/P ratio of 5 [123-125]. Abovementioned facts give us insight about possible synergistic effect which might be obtained with respect to disruption of mitochondrial functions. PEI also provides nanoparticles the ability to deliver gene and protein/peptides for combination drug therapy. PEI and its variants are amongst the most studied cationic polymers for delivery of nucleic acids. It has a high charge density at reduced pH values due to presence of nitrogen atom at every third position along the polymer which imparts PEI the ability to promote in vitro and in vivo gene transfection [126]. Recently, many researchers have

changed the conventional gene delivery strategy by incorporating surface modifying agents by either conjugation on PEI backbone or by other chemical methods in form of peptides or antibodies for tumor specific gene delivery. Shen et al. reported incorporation of high mobility group box 1 (HMGB1) containing nuclear localization sequences (NLS) along with gene with the aim of enhancing nuclear delivery of pDNA. The results demonstrated 4 folds increase in transfection efficiency [127]. Similarly, Tat peptide, tumor specific peptide and many more have been reported to enhance the transfection efficiency in comparison to plain PEI mediated gene delivery [128]. For targeting pDNA to adipose tissue, nuclear localization signal (NLS) peptide of Simian vacuolating virus 40 large T-antigen was conjugated to PEI which demonstrated robust and sustained expression of exogenous genes in adipose derived cells [129]. The conjugation of PEI with metals has opened a new avenue in field of PEI based systems. Such systems have demonstrated to provide diagnostic as well as imaging capability of PEI based systems in addition to therapeutic ability. The result obtained for magnetic PEI vectors have shown to be effective in monitoring and imaging the targeting of PEI based vectors. Such system can enhance the targeting efficiency as well as help to study the cellular internalization. Li and coworkers fabricated PEI based theranostic nanoparticles using iron oxide nanoparticles [130]. Magnetic iron oxide-based nanoparticle comprising a magnetic inner core and a disulfide-containing polyethylenimine (SSPEI) outer layer was synthesized, for redox-triggered gene release in response to an intracellular reducing environment. The results of this study demonstrated the potential of a disulfide-containing PEI-decorated magnetic nanoparticle as highly potent and low-toxic theranostic nano-system for specific nucleic acid delivery inside cancer cells. Similar work had been reported using 3-(2-aminoethylamino) propyltrimethoxysilane modified magnetic nanoparticles [131]. These modified magnetic nanoparticles were further conjugated to PEI-folic acid (PF) conjugate. The formed theranostic nanoparticles demonstrated feasibility as contrast agents in magnetic resonance imaging (MRI) and as gene carriers for gene delivery. Apart from being contrast agent, these nanoparticles demonstrated specific cellular uptake by KB cells usingWI-38 cells as comparison by confocal microscopy. Mesoporous silica nanoparticles were functionalized with cyclodextrin grafted PEI. Pyruvate kinase M2 isoform (PKM2), (which is overexpressed in several cancer types), was used as a target gene to evaluate the effectiveness of developed mesoporous silica nanoparticles based delivery system [132]. Cellular internalization, subcellular localization, gene silencing capability

and anticancer activity of siRNA-loaded nanoparticles were assessed with MDA-MB-231 human breast cancer cells. These results demonstrate that PEI can be used as potential surface modifying agents owing to its unique properties supporting drug delivery as well as targeting.

1.9. Lenalidomide

Lenalidomide was initially intended for treatment of multiple myeloma for which thalidomide is an accepted therapeutic treatment and shares structural similarity with it. In the recent time, lot of research has been done for using this drug in brain tumor. Clinical trials conducted on this drug give an insight for use of this drug in treatment of brain tumors with phase II clinical trials still going on [133, 134]. This makes it a potential candidate for treatment for brain tumor in combination with other therapeutics. The drawback of lenalidomide is that it is not able to cross blood brain barrier and reach CSF at therapeutically relevant concentrations. It also accompanies atypical bio-distribution and very short residence time in body. Lenalidomide suffers low bioavailability along with atypical bio-distribution. One of the major disadvantages of this drug is anemia. Results of clinical trials on human subjects revealed that administration of lenalidomide causes Warm Autoimmune Hemolytic Anemia (AIHA) [135]. This is due to significant hemolysis of the patient's own red cells. Oral administration of drug lead to bile acid malabsorption induced diarrhea [136]. Hence it needs to be administered in such a way to enhance its brain uptake and residence at tumor site.

1.10. Aims and Objectives

The aim of the present research work was design and fabrication of polyethylenimine based theranostic nanoparticles for targeting brain tumor. The proposed study was planned to achieve an effective and selective brain tumor targeting using theranostic nanoparticles in order to serve the dual purpose of diagnosis and therapy of tumors using a single nanoparticulate system that will help inhibit the growth of tumor by targeting therapeutic moiety to tumor (sub cellular compartment) so as to prevent metastasis and growth of tumor. The fabricated nanoparticles would further possess an additive effect of hyperthermia for effective tumor therapy. The targeting will help to reduce the toxicity associated with anticancer therapeutic moieties and will explore metal based novel theranostic nano-platform for targeting brain tumors.

1.11. Objectives of Proposed Work

- Synthesis of Iron and Platinum based alloy nanoparticles (FePt) for drug delivery.
- Fabrication of Polyethylenimine modified FePt based nanoparticles (Theranostic nanoparticles).
- Fabrication of pH responsive drug alloy nanoconjugates.
- Exploration of Polyethylenimine (PEI) modified FePt based theranostic nanoparticles for delivery of therapeutic moiety.
- Radiolabeling of formulation using suitable radioisotopes, their characterization and biological evaluation.
- Evaluation of efficacy of selected system in delivering the therapeutic moiety at the target site by in-vivo radioactivity distribution studies.
- Study sub cellular localization of developed theranostic nanoparticles.
- Exploration of theranostic nanoparticles as platform for simultaneous therapy and imaging of brain tumor.

1.12. Hypothesis

It was hypothesized that synthesized FePt based theranostic nanoparticles will inhibit growth of tumor via. multiple approaches (Iron will cause hyperthermia and it will induce production of ROS via Fenton reaction. pH responsive drug release through Hydrazone bonding will protect premature release of drug. Drug will inhibit tumor growth owing to

its anti-angiogenesis activity and after cellular co-localization nanoparticles will hamper mitochondrial function) which together give synergistic affect leading to specific and effective treatment of brain tumor. Synthesized FePt based nanoparticles will act as potential platform for multimodal imaging and therapy of brain tumor.

1.13. Plan of Work

1. Procurement of chemicals and therapeutic moiety.
2. Synthesis of Iron-platinum alloy nanoparticles.
3. Optimization of Iron-platinum alloy nanoparticles (metal core).
4. Loading of therapeutic moiety on coated metal core.
5. Characterization of synthesized metal nanoparticles viz. Elemental analysis, Infrared spectroscopy, confocal microscopy, magnetic susceptibility, hyperthermia etc.
6. Synthesis of polymer drug conjugate.
7. Characterization of modified polymer and metal nanoparticles.
8. *In vitro* cell line studies.
9. Development of strategy for radiolabeling, selection of suitable BFCA.
10. Standardization of radiolabeling procedures and characterization of radiolabeled formulation.
11. *In vivo* studies in laboratory animals.
12. Scintigraphy studies using radiolabeled formulation.

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