2.0 Brain tumor

Brain and central nervous system (CNS) tumors are a heterogeneous group of diseases and include tumors of the brain, cranial nerves, spinal nerves, spinal cord, and the meninges. These tumors comprise more than 100 histologic types as per World Health Organization (WHO) Classification of Tumors of the Central Nervous System based on cell of origin and other histopathologic features. Brain tumors can be broadly classified into two categories: 1) malignant and 2) nonmalignant (or benign) tumors. Most brain and CNS tumors that are diagnosed in the United States are nonmalignant (Figure 2.1), and most of these are nonmalignant meningiomas (1).

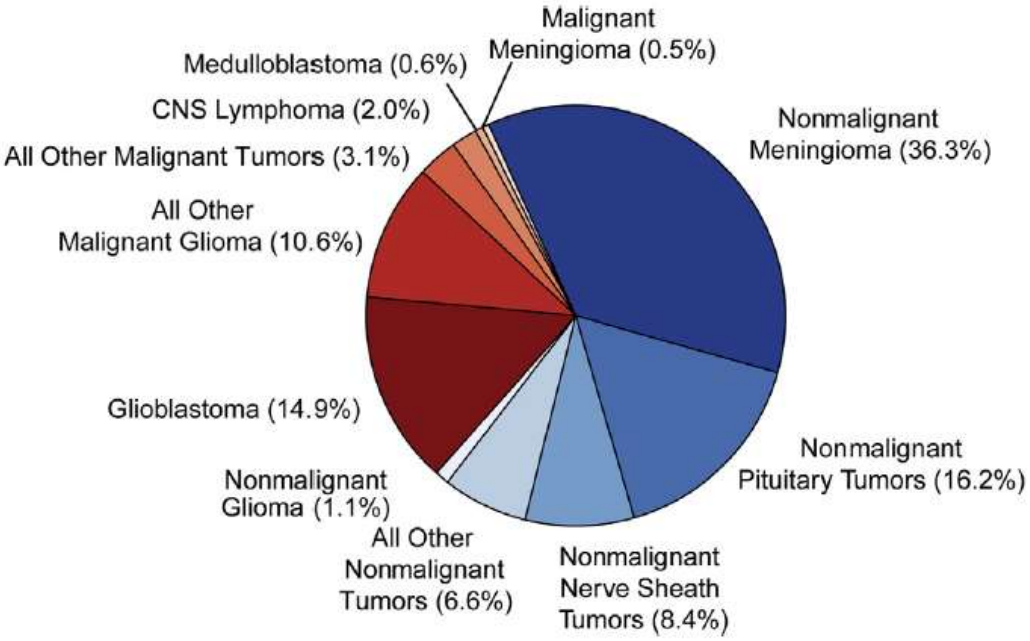


Figure 2.1: Distribution of primary brain and other CNS tumors by behavior and histology

The WHO classified brain tumors based on their morphology, cytogenetics, molecular genetics, and immunologic markers into various types. The WHO also classified grading system for brain tumors according to their histology and slow-growing and fast-growing properties (Figure 2.2) (2). Primary brain tumors are named according to the type of cells or the part of the brain in which they begin (Figure 2.3).

WHO Grade	Criteria
I	• The tumor cell has a low proliferative potential, and looks similar to a normal cell • The proliferation is slower than grades II, III, and IV tumor cells • There is a possibility of cure by surgical resection alone
II	• The tumor cell has a higher proliferative potential than grade I tumor cells with less mitotic activity • Proliferation and metastasis are slower than grade III and IV tumor cells • The possibility of recurrence after surgery and becoming a higher-grade tumor is greater than grade I tumor cells
III	• The tumor cell has a higher proliferation potential than grade I and II tumor cells and looks very different from normal cells • Marked histopathological changes with an increased mitotic activity and highly metastatic in nature • Treated with aggressive adjuvant chemotherapy
IV	• The tumor cell has a high proliferation potential with postoperative progression and fatal outcomes • The tumor cells are mitotically active and necrosis-prone • Grade IV tumors cannot be cured, however, they are treated with aggressive adjuvant chemotherapy

Figure 2.2: WHO Tumor Grading System Based on Histology and Proliferation Potential

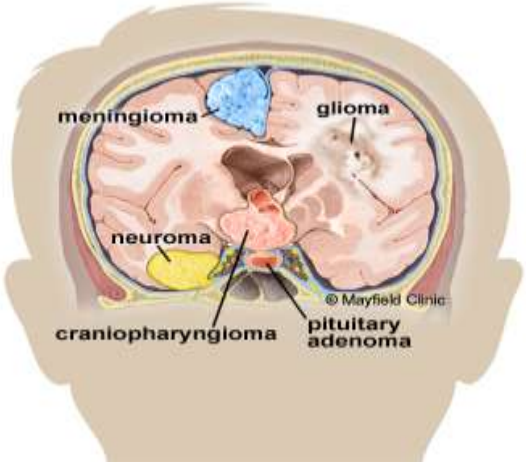


Figure 2.3: Different types of primary brain tumors

2.1 Current treatment approaches for brain tumor

Multiple treatment approaches have been experimented for treatment of brain cancer and may vary depending on the type, grade, size and location of the tumor; whether it has spread; and patient age and general health. The multidisciplinary approach for treatment of brain tumor includes combination of surgery, radiation and chemotherapy(3). However, none of these treatments, alone or in combination, is considered efficient in managing this disease, resulting in a survival of less than 15 months (4).Conventional chemotherapy has its drawback of lower concentration of drug at site of action and also imparts side effects to normal cells. Furthermore, conventional therapy also failed to overcome the barriers raised due to tumor microenvironment acidosis (low pH), enhanced permeability retention, hypoxia (low oxygen level) and extensive angiogenesis (highly vascularised). It can be easily removed by reticulo-endothelial system (RES) and renal clearance. In case of surgery, the tumors can be removed at its early stage; however, if the tumor gets converted into malignant, then the treatment is troublesome, costly and probably inefficient. Radiotherapy may lead to radio-dermititis after or during treatment.(5)

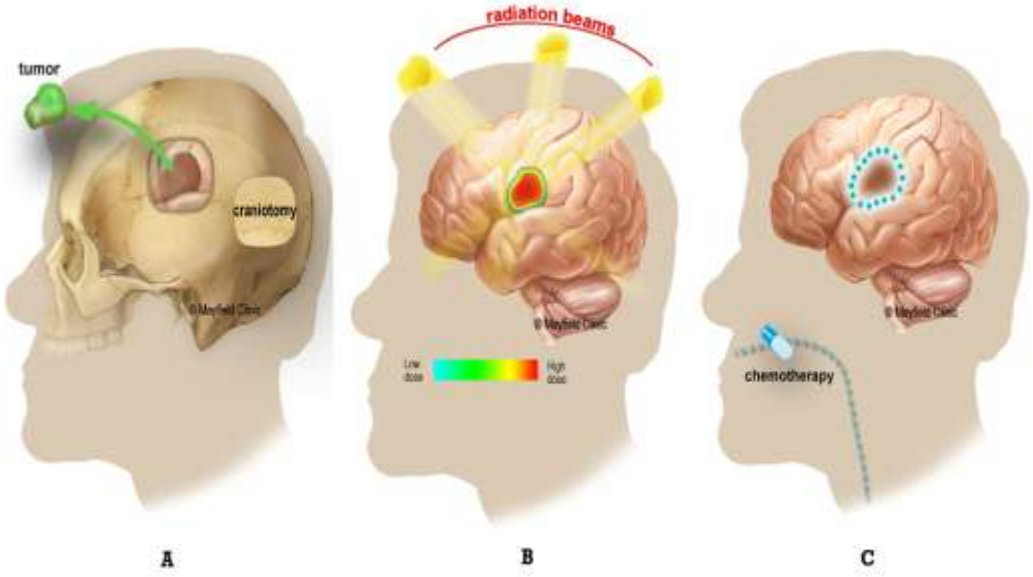


Figure 2.4: Treatment approaches of Brain tumor- (A) Surgery, (B) Radiation and (C) Chemotherapy

2.2 Hurdles in drug delivery to brain

2.2.1 Blood brain barrier

The blood brain barrier (BBB) is a specialized system composed of five major parts: endothelial cell layer of capillaries, pericytes, immune cells, astrocytes, and basement membrane collectively known as the neurovascular unit (NVU) (Figure 2.5). This NUV is a fundamental to the physiological role of the BBB and its perturbation in pathological states (6). This BBB prevents transportation of approximately 98% of the small molecules and nearly 100% of large molecules including recombinant proteins and genes into the brain and reaching the tumor sites.The BBB act as a physical (tight junctions), metabolic (enzymes) and immunological barrier which strictly limits drug transport into the brain (7–9).

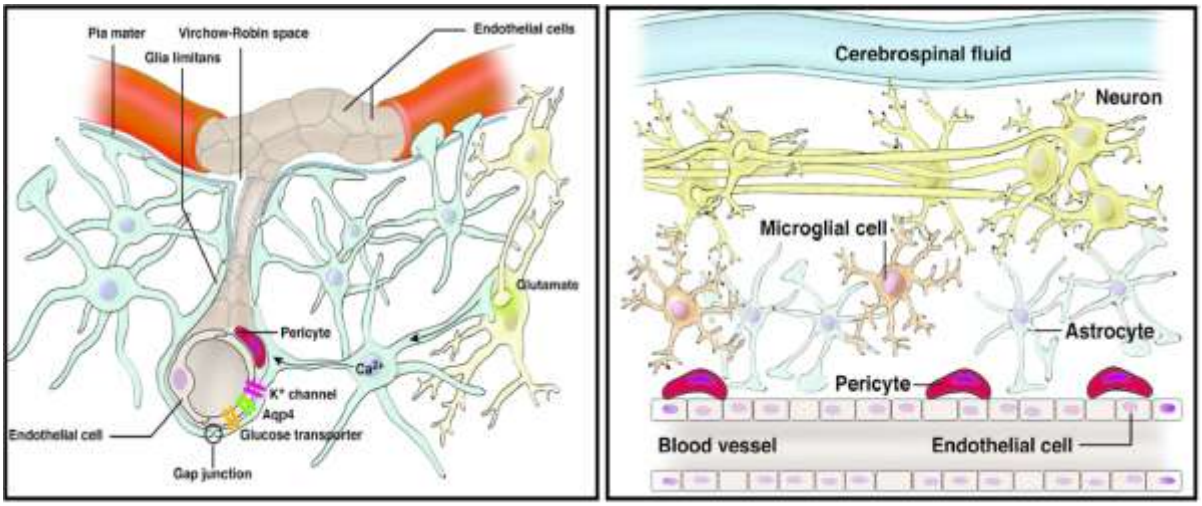


Figure 2.5: Blood brain barrier

At the molecular level, the BBB comprises tight junction proteins, adherence proteins, transporters, basal lamina, and extracellular matrix. The tight junctions and adherens proteins prevent paracellular diffusion, so drugs entering the brain parenchyma generally must cross the luminal and abluminal plasma membranes of the endothelial cell. While most small lipophilic substances may cross the BBB by simple diffusion, other potential processes include facilitated diffusion, simple diffusion through an aqueous channel, active transport, or paracellular diffusion (10). Different transports mechanisms involve in passage of drugs across BBB are summarized in figure 2.6 (2). BBB permeability is, at least in part, controlled by intra- and intercellular

signaling among the endothelial cells and surrounding astrocytes and neurons (11).The transporters present in BBB control the transport of necessary water-soluble molecules (such as glucose) and large molecules (e.g., some proteins).So for the transportation of drugs into or out of CNS, the affinity of drug for its carrier/transporter plays critical role (for those agents requiring specific transporters).

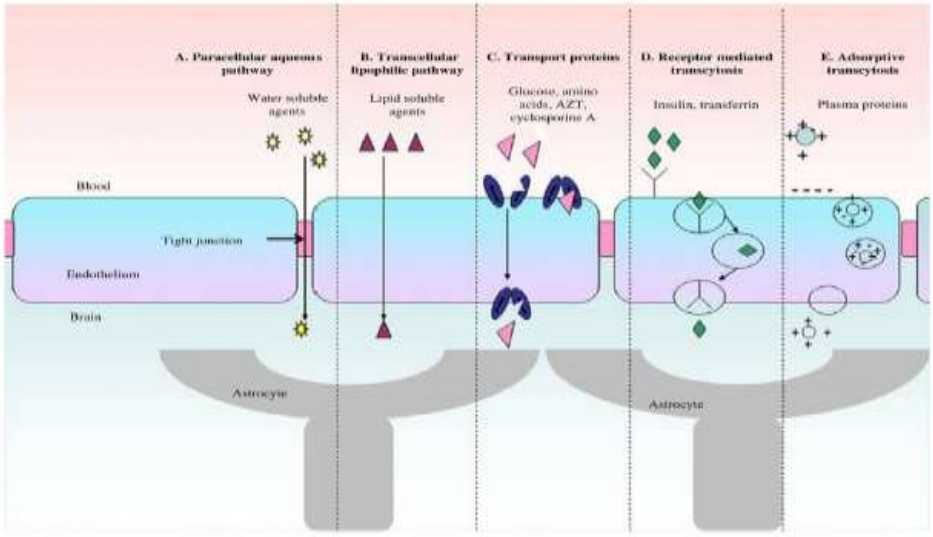


Figure 2.6: Different transport mechanisms involve in passage of drugs across BBB

Uptake/influx transporters, which facilitate entry *into* the brain, include organic anion transporting polypeptides, nucleoside transporters, monocarboxylate transporters, and peptide transport systems (12). Active *efflux* transporters are also present at the BBB (figure 2.7) and serve to restrict entry of many chemotherapeutic agents into the CNS. Efflux transporters include P-glycoprotein (Pgp), breast cancer resistance proteins, and multidrug resistance proteins. There is an association between polarity and active efflux at the BBB, with increased interaction of drug efflux transporter protein with agents that are able to form a greater number of hydrogen bonds (13).

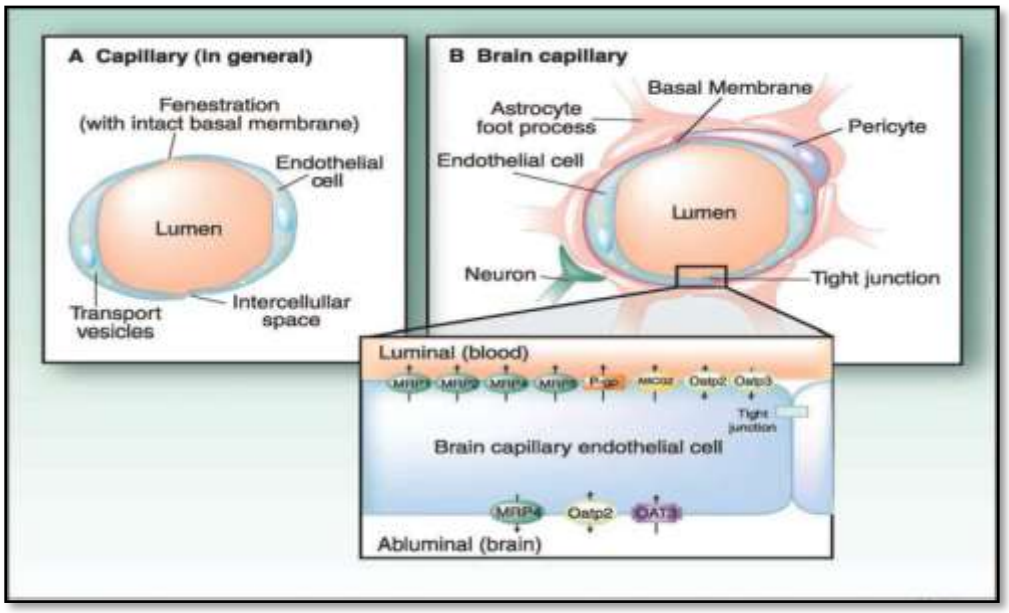


Figure 2.7 Efflux transporters expressed on BBB

2.2.2 Blood- brain tumor barrier

The blood–brain tumor barrier (BBTB) is located between micro vessels formed by highly specialized endothelial cells (ECs) and brain tumor tissues. This BBTB limits the paracellular delivery of most hydrophilic molecules to tumor tissue. BBTB is formed after rupture of BBB by tumor cell clusters (grown to a certain volume) (10,14). BBTB becomes the main obstacle of drug delivery with the worsening of brain tumors, angiogenesis and gradual impairment of BBB. At this stage, tumor neo vasculature has formed to support the growth of gliomas. The abnormality of micro vessels enhances the permeability of BBTB, whereas the cranial microenvironment and/or the gliomas specificity make malignant gliomas less permeable (15,16). Even though the BBB is compromised in malignant gliomas, the permeability differs from other regions. The infiltrating gliomas, especially around the tumor edge, still utilize the available brain vasculature and the BBB still limits glioma-targeted transport of chemotherapeutic agents (17).

2.2.3 Blood-CSF barrier

Blood-CSF barrier (BCSFB) is another barrier formed by modified epithelial cells rather than endothelial cells and located at the choroid plexus and the arachnoid membrane. This BCSFB

also blocked the passage of systemically administered therapeutic agents (18). The CSF has its own characteristics which play important roles in drug delivery and can affect delivery of drug from the CSF into the CNS tissue: (a) Even though CSF flows, drugs are not homogeneously mixed throughout and circulate inhomogeneously; this circulation can be affected by gravity, presence of increased proteins or tumor; (b) Continuous CSF production leads to dilution of administered drugs; (c) Elimination of some drugs from CSF into bloodstream or glymphatic system or metabolism may also take place (10).

2.3 Circumventing barriers for effective drug delivery

Over the past two decades, number of clinical trials and investigations of cytotoxic agents, molecularly targeted agents, biologic response modifiers, and immunomodulatory agents have increased for treatment of brain tumors. Along with this various techniques also have been explored to overcome the barriers associated with the therapy of brain tumors which are summarized in table 2.1.

Table 2.1: Techniques to overcome BBB barrier (Adopted from Warren KL 2018)

Technique	Advantage	Disadvantage
High-dose systemic chemotherapy	Higher *C*max in circulation may result in higher *C*max in central nervous system (CNS) (assuming linear increase in BBB penetration)	• Toxicity • If threshold for BBB penetration is reached, toxicity is increased without increasing chance of benefit • If no drug penetrates at low dose, unlikely to achieve drug penetration at higher doses
BBB disruption	Temporary increase in BBB penetration into CNS	• Toxicity • Not tumor specific •Unknown exposure (adequate concentration over adequate time periods)
Inhibition of drug efflux transporters	Block drug efflux from BBB allowing increased CNS penetration	• Toxicity • Results in increased plasma drug levels due to decreased drug clearance (P-glycoprotein

		inhibitors not specific for BBB)
Intraarterial delivery	Higher drug concentrations in region supplied by artery ONLY during first pass through tumor	•Streaming effect, inhomogeneous delivery (toxicity, insufficient delivery) • Unable to reach tumor cells outside of area supplied by artery • Once drug enters systemic circulation, no longer any PK advantage
Convection-enhanced delivery	Bypasses BBB; direct installation into tumor bed	• Invasive procedure • Difficult to reach all tumor cells • Need to ensure adequate exposure of active agent for a long enough period of time but difficult to evaluate PK

Radiation therapy, osmotic disruption, focused ultrasound, and pharmacologic manipulation by bradykinin and its agonists are each associated with increased permeability of the BBB, but their effects are non-specific and time limited (19). All these therapies have pros and cons. The conventional treatment has often struggled to resolve tumor microenvironment obstacles such as enhanced permeability and retention (EPR) effect, hypoxia, acidosis and extensive angiogenesis (10). Conventional therapy also possesses disadvantages viz. non-specific distribution of drug which may lead to toxicity to normal cells, generating resistance and also impart unwanted side effects (6). Conventional therapeutic carrier can be removed by RES or by renal excretion easily (7). Such disadvantages prompt the fabrication of nano-delivery system that can particularly target therapeutic moieties to tumor cells along with controlled release and targeting of therapeutic moieties to enhance intracellular localization, resulting in a reduction in non-specific toxicity of cells. Various strategies are had been recognized for therapeutic delivery to tumor sites (10). The main priority in the production of therapies for cancer treatment is drug delivery which can target cancer which can provide therapeutic concentrations of anticancer therapeutic moieties at the action site and avoid toxicity to normal healthy cells.

2.4 Nanoparticles as drug delivery platform

Keeping in mind the drawbacks of conventional therapy, the development of novel and smart delivery systems for efficient treatment of brain tumor, have attracted many researchers. Nanotechnology-based delivery systems are extensively studied in efficient treatment of brain tumors. Nanoparticles have many advantages such as target specific thus reducing toxicity to normal cells, higher loading capacity thus reduction in dose, controlled release thus reducing dose frequency. Several nanoparticles with distinct biological functions have been fabricated for cancer treatment over the past three decades by modulating their physicochemical properties including composition, size, shape and surface modification. Two clinically successful targeted therapies viz. antibody-drug conjugates and nanoparticle based systems have emerged among the nanoparticles. Three antibody-drug conjugates and seven drug delivery systems based on nanoparticles targeted at a variety of human cancers have been approved at the moment(20). In addition, nanotechnology provides encouraging prospective for screening, formulating and administering drugs which were formerly limited due to solubility. Nonetheless, the majority of nanoparticles applied for treatment of cancer did not meet the approval of Food and Drug Administration (FDA) due to development of drug resistance, tumor relapse and failure to produce an improved anti-cancer efficacy or diagnosis and targeting. There are several nanoparticles such as protein-based nanoparticles, lipid based nanoparticles, polymeric nanoparticles and metal-based nanoparticles investigated in the treatment of cancer (21,22).However, surface functionalization of nanoparticles with biomolecules (targeting moiety) can be a promising approach for effective targeting. Targeting moieties such as proteins, peptides, antibodies, aptamers can recognize tumor associated or tumor specific antigens in microenvironment of tumor. Ligands viz. proteins, peptides and sugars are typically more desirable than antibodies because of higher purity, higher stability, non-immunogenicity and easy to fabricate with synthetic process (23).

Recently, protein-based nanoparticles have been extensively investigated owing to their unique properties viz. ability to deliver nucleic acid, genes, peptides and proteins, both lipophilic and hydrophilic therapeutic moieties can be easily delivered, fabrication procedure is easy, during storage they show greater stability, more target specific when surface functionalized with

targeting moiety and finally they are biocompatible and safe as compared to other nanoparticles (24,25). Amongst protein-based nanoparticles, albumin was selected as carrier for exploring its utilization in the treatment of cancer.

2.5 Albumin based nanocarriers

Albumin is macromolecule and mostly available in body as a plasma protein (35-50 gm/L). It is synthesized in liver with an approximately rate of 0.7 mg/h for every gm of liver (10-15 gm daily)(26,27). It is biocompatible, biodegradable, non-immunogenic, stable, easy to purify, water-soluble and non-toxic (28). Again there are several types of albumin such as ovalbumin, human serum albumin and bovine serum albumin (BSA). Amongst them, BSA was considered for the research owing to their low cost, easily purified, biocompatibility, biodegradability, unusual ligand binding properties and non-toxic as compared to ovalbumin and rat albumin and BSA are widely accepted in commercial market in pharmaceutical industries (29). Albumin nanocarriers have been long used as delivery systems for drugs and genes. The biodegradable, nontoxic, and non antigenic properties of albumin in addition to its easy functionalization, make albumin nanocarriers ideal candidates for enabling specific tumor targeting(4). Different albumin based carrier systems are depicted in figure 2.8.

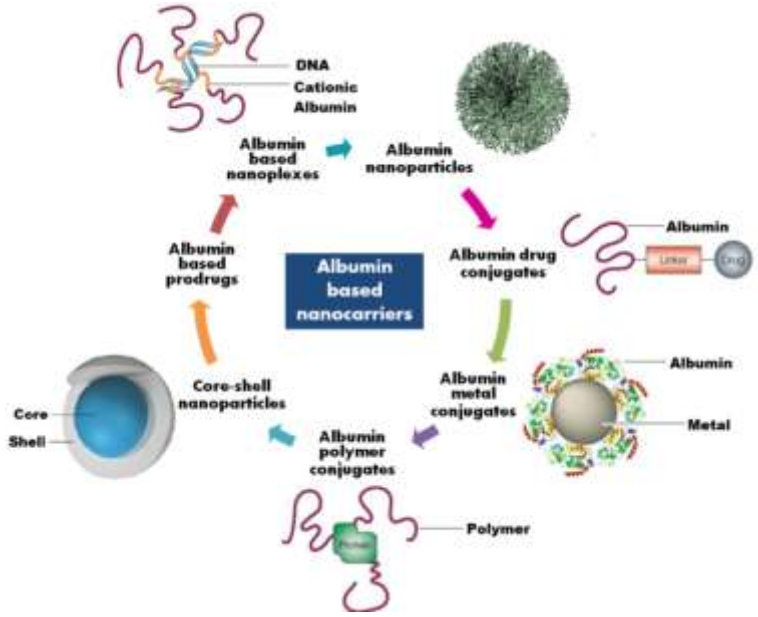


Figure 2.8 Albumin based nanocarriers

2.5.1 Albumin nanoparticles

Albumin based nanoparticles are utilized for cancer treatment as they are biodegradable, non antigenic and can be also surface modified which may help in avoiding the undesirable toxicity of drugs by modifying their body distribution and improve their cellular uptake. They also have targeting potential because proteins themselves act as passive as well as active targeting moiety. Other targeting ligands can also attach in these carriers to provide site specificity (25). Albumin NPs have been successfully utilized for targeted delivery of drugs to the brain tumors. Albumin-binding proteins SPARC (secreted protein acidic and rich in cysteine) and glycoprotein 60 (gp60) are highly expressed on human glioma cells. In contrast, normal BBB capillaries show a very low level of albumin-binding proteins, resulting in poor permeation of native albumin. Two consecutive steps are essential for glioma drug delivery: targeting and penetration. Albumin not only serves as a targeting ligand to albumin-binding proteins but also induces the transcytosis across BBB and endocytosisinto glioma cells (4).

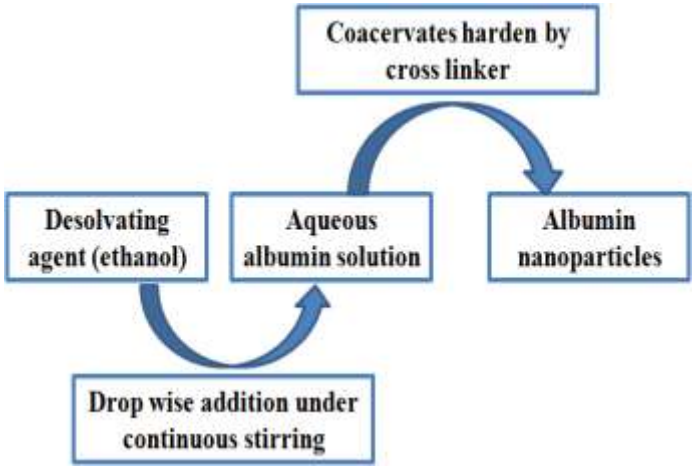
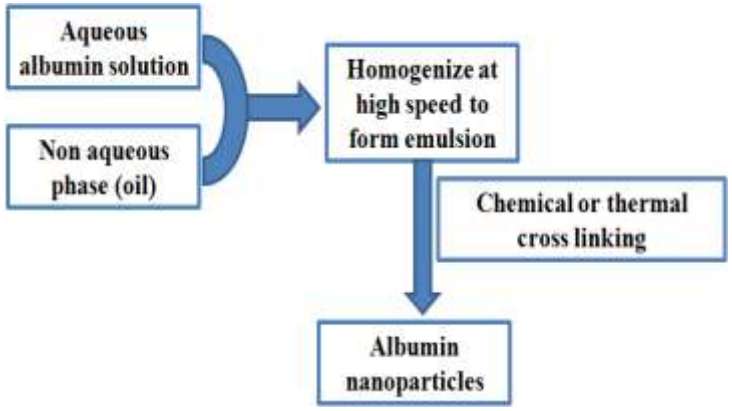
2.5.2 Method of preparation of albumin nanoparticles

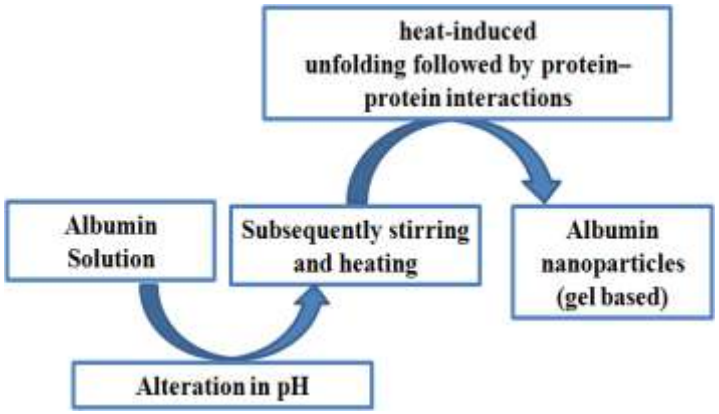
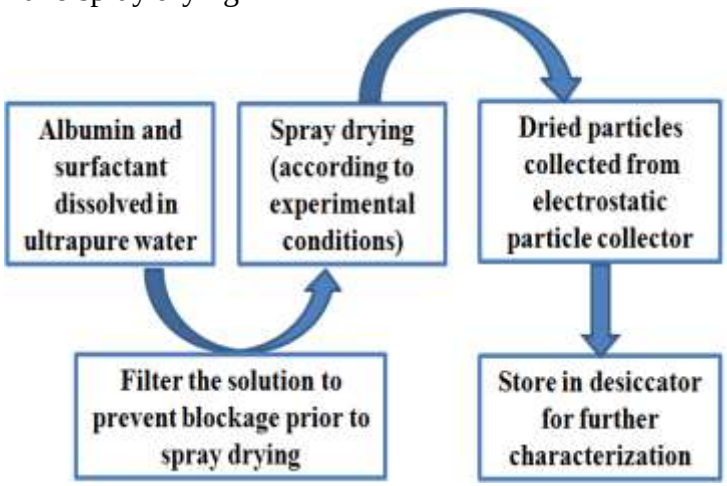
Albumin nanoparticles can be prepared by several methods like desolvation, emulsification, thermal gelation, nano spray drying, nab technology and self-assembly etc. All preparation methods are summarized in table 2.2. The selection of the method is based on several factors such as type of system, area of application, required size, type of drug (hydrophilic or hydrophobic), etc.

2.5.3 Applications of albumin nanoparticles in cancer therapy

Albumin based nanoparticles are widely explored protein based nanocarriers for cancer therapy. Several categories of drugs can be encapsulated in albumin via covalent conjugation, electrostatic interaction or hydrophobic interaction. This makes albumin a versatile drug delivery carrier. Apart from this, its accumulation in the tumor also makes it suitable candidate for cancer therapy. Different albumin based nanocarriers and their applications in cancer therapy are summarized in table 2.3

Table 2.2: Method of preparation of albumin nanoparticles (Adopted from: Kudarha et al 2017) (25)

Sr. No.	Method	Key factors/ variables	Advantages	Disadvantages
1.	Desolvation 	• Albumin concentration • pH of albumin solution • Aqueous phase: Desolvating agent volume ratio • Rate of addition of desolvating agent • Amount of cross linker • Stirring speed • Stirring time • Solvent polarity	• Most accepted method • Robust • Reproducible • No surfactant required • Used for encapsulation of hydrophilic molecule.	• Chemical cross linking agent may cause toxicity • Require removal of organic solvent
2.	Emulsification 	• Albumin concentration • Aqueous phase: oil phase volume ratio • Rate of addition of emulsion • Emulsification time • Speed of homogenizer • Heating temperature for thermal stabilization • Amount of surfactant	• Hydrophobic drugs can be entrapped • Both chemical as well as thermal stabilization methods can be used for stabilization of nanoparticles	• Require organic solvent • Require removal of oily residue and surfactant • Chemical cross linking agent may cause toxicity • Require high temperature for crosslinking • Larger particle size than desolvation

		• Type of surfactant • Amount of cross linker		method
3.	Thermal gelation 	• pH • temperature • Protein concentration • Ionic strength • Nature and concentration of other solids	• Used for fabrication of nano hydrogels	• Thermolabile drugs cannot be used • Require high temperature
4.	Nano spray drying 	• Albumin solution concentration • Surfactant concentration • Drying air flow rate • Inlet temperature • Spray mesh size • Organic solvent content of spray solution	• Single step • Continuous and scalable method • Final drying not required • High reproducibility • High encapsulation efficiency • Cost effective • Also overcome biopharmaceutical disadvantage of drug	• Large particle size • Higher processing time • Highly viscous polymer solution can't be use because of small diameter of orifice of spray nozzle • Crust may contaminate fine particles • Mechanical shear can alter the properties of shear

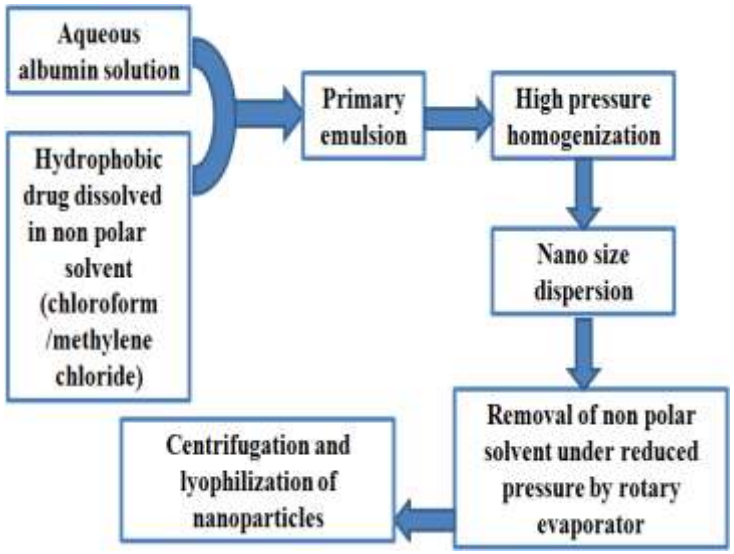
				sensitive substance • Require hightemperature
5.	Nab-technology  	• Albumin solution concentration • Aqueous phase: organic phase volume ratio • Homogenization time • Homogenization cycle • Homogenization pressure	• No surfactant required • No denaturation of albumin • High loading of poorly water soluble drugs • Nanoparticles formed are safe and suitable for intravenous use of hydrophobic drug	

Table 2.3:Application of albumin nanocarriers in cancer therapy (Modified from: Kudarha et al 2017) (25)

Albumin nanocarrier	Targeting ligand	Preparation method	Size	Morphology	Drug	Cell line	Study Outcome
Brain tumor							
Peptide functionalized albumin nanoparticles	Cell penetrating peptide (cRGD and KALA)	Self assembly	241 nm	Spherical nanoparticles	Doxorubicin (DOX)	U87-MG glioblastoma cells	Better tumor targeting, cell penetrating, and endolysosomal pH-responsive properties.
PEG grafted BSA nanoparticles	Lactoferrin	Desolvation	150-155 nm	nanoparticles	DOX	BCECs and C6	Better permeation enhancement property, increased dual targeting effect of nanoparticles for brain delivery and facilitated the uptake of DOX in the brain tissue
HSA nanoparticles	-	Desolvation	80–90 nm	nanoparticles	Imatinib base (IMTb)	U87-MG glioblastoma cells	Cytotoxic effect of the IMTb loaded HSA nanoparticles is higher than that of free IMTb for glioblastoma
HSA nanoparticles	Cationic albumin and mannose	High pressure homogenizatio n	90.5 nm	Spherical nanoparticles	DOX	bEnd.3 cell, and U87 MG glioblastoma cells	Better tumor targeting
Albumin lipid nanoparticles	-	Emulsification solvent evaporation	110.1 nm	Core shell nanoparticles	Docetaxel (DXT)	U87 MG, A549, bEnd.3, HUVEC, BMEC cells	Lower toxicity and a superior anti-glioma effect as compare to standard DTX preparations.
Cationic albumin	-	(o/w) emulsion	57.7 nm	nanoparticles	Aclarubicin (ACL)	C6 glioma cells	Higher accumulation of cationic albumin conjugated

conjugated pegylated nanoparticles		technique					nanoparticles in tumor mass as compare to non-conjugated nanoparticles with better retention, Survival time also increased.
Breast Cancer							
HSA Nanoparticles	-	high pressure homogenizatio n	156.9 ± 3.2 nm	nanoparticles	Pirarubicin (THP) and Paclitaxel (PTX)	4T1 cells	Increased apoptosis and G2/M cell cycle arrest against 4T1 cells and significantly lower side effects regarding bone marrow suppression and organ and gastrointestinal toxicities.
HSA Nanoparticles	-	Nab technology	137.3 nm	nanoparticles	Lapatinib	SKBr3 cells	better inhibit HER2 phosphorylation of tumor cells and exhibited superior anti-tumor efficacy in tumor-bearing mice with no subchronic toxicity
HSA Nanoparticles	-	Nab technology	147 nm	nanoparticles	DXT	MCF7 cells	DTX nanoparticles showed dose and time dependent cytotoxicity in MCF-7 cells. Real-time PCR analysis showed higher alteration of pro-apoptotic gene expression in MCF-7 cells after treatment with DTX nanoparticles compared with free DTX.
Methotrexate –human serum albumin	luteinizing-hormone releasing hormone	MTX firstly conjugated with HSA by using EDC	120.5 nm-138.56 nm	nanoparticles	Methotrexate (MTX)	4T1 breast cancer cells	Significant tumor growth delay were observed in4T1 tumor bearing mice treated with LHRH targeted MTX–HSA

nanoparticles	(LHRH)	and NHS and this conjugate is further cross-linked by EDC to form nanoparticles					NPs compared to non-targeted MTX–HSA NPs treated group. The body weight loss of LHRH targeted nanoparticles treated groups was very low.
HSA nanoparticles	Biotin/ Folate	Emulsification method	185 nm and 205 nm	Drug- HSA conjugated nanoparticles	DXT	MDA-MB-231, A549 and 4T1 cells	Conjugated nanoparticles more significantly reduce tumor size and increases survival rate of animal compared to free drug.
PEI-enhanced HSA nanoparticles	-	Desolvation	137 nm	nanoparticles	DOX	MCF7 cells	Coating of cationic polymer over the HSA nanoparticles improved the cell penetration and showed more potent cytotoxic effects on MCF 7 cells.
Cervical Cancer							
Aptamer Functionalized Cisplatin-Albumin Nanoparticles (Apt-Pt NPs)	EGFR RNA Aptamer	Desolvation	40 nm	nanoparticles	Cisplatin	Human Hela cell line	Enhanced intracellular cisplatin release rather than extracellular and avoid unnecessary accumulation of drug in body.
Colon Cancer							
HSA nanoparticles	Folate	Emulsification	165.6 ± 15 nm	nanoparticles	Curcumin	-	Nanoparticles showed sustained release of Curcumin and *in vivo* study showed prolonged retention time of drug and better antitumor activity.
Prototype of HSA	-	Self assembly	267.3 nm	nanoparticles	DOX	HCT 116 and A549	excellent tumor target ability probably due to gp60-mediated

nanoparticles						cells	transcytosis mechanism
HSA nanoparticles	-	Nab technology	60~120 nm	nanoparticles	DOX and TRAIL protein	HCT116 cells	Co delivery of DOX and TRAIL offered potential synergistic apoptosis-based anticancer therapy
Gastric Cancer							
HSA nanoparticles	-	BSA cross linked by Schiff base-containing vanillin to form nanoparticles	100.5 nm	nanoparticles	DOX	BGC-823 cells	Formulation showed superior extension of survival time than free DOX and DOX-BSA-NPs, and greater tumor suppression than free DOX.
Liver Cancer							
BSA nanoparticles	glycyrrhizic acid	Emulsification (High pressure homogenization)	157.5 nm	nanoparticles	10-hydroxycamptothecin	SMMC7721 cells	promising new vehicle for hepatocellular carcinoma-targeting therapy
Recombinant HSA Nanoparticles	Glycyrrhetinic Acid (GA)	Desolvation	170 nm	nanoparticles	DOX	HepG2 cells	The targeted NPs exhibited higher cellular uptake in a GA receptor-positive liver cancer cell line than non-targeted NPs and biodistribution experiments showed that targeted NPs exhibited a much higher level of tumor accumulation than non-targeted NPs
Albumin nanoparticles	glycyrrhetinic acid	Desolvation	258.8±6.4 nm	nanoparticles	Curcumin	HepG2 cells	NPs were significantly more cytotoxic to HepG2 cells and in a concentration-dependent

							manner.
BSA nanoparticles	Hematoporphyrin (HP)	Desolvation	372.6±10.9 nm	nanoparticles	DOX	HepG2 cells	Cellular uptake from HP-NPs was proportional to the expression level of LDL receptors on the cells, indicating possible involvement of LDL receptor-mediated endocytosis (RME) in uptake.
Lung Cancer							
Self-assembled albumin nanoparticles	-	Self assembly	~340 nm	nanoparticles	DOX and TRAIL protein	H226 cells	Improved anti-tumor efficacy was found to be due to the synergistic apoptotic effects of DOX and TRAIL
Albumin nanoparticles	-	Emulsion-evaporation cross-link method	163-169 nm	nanoparticles	DXT	A549 cells	Pegylated nanoparticles showed higher cellular uptake and superior antitumor activity as compare to non pegylated nanoparticles.
Multi Drug Resistance (MDR) cancer							
TPGS modified reduced BSA nanoparticles	-	Ultrasonication	173-338 nm	Albumin-polymer conjugated nanoparticles	PTX	MCF-7 and MCF-7/ADR cells	Polymer- protein conjugate based nanoparticles showed higher drug loading and entrapment of lipophilic drug and *in vitro* revealed its ability to overcome MDR by inhibiting P-gp efflux.
BSA nanoparticles	-	Self assembly	50 nm	nanoparticles	DOX and Verapamil (VER)	HCT-15 cells and 293T cells	As compared with free DOX and DOX/BSA nanoparticles, DOX/VER/BSA nanoparticles exhibited a stronger tumor cell inhibitory effect because of the enhanced intracellular DOX

							concentration caused by the efflux pump inhibition of VER.
Ovarian Cancer							
BSA nanoparticles	-	Modified desolvation	10-200 nm	nanoparticles	Albendazole (ABZ)	SKOV3, OVCAR3, and HOSE cells	BSA-ABZ may hold promise for the treatment and control of progression of ovarian cancer with ascites. However further studies are also required.
Pancreatic Cancer							
HSA nanoparticles	-	Nab technology	150±27 nm,	nanoparticles	Gemcitabine (GEM)	BxPC-3 cell	The enhanced *in vivo* efficacy of GEM-HSA-NPs toward the pancreatic cancer cell line suggests their potential role for use in the clinical field.
HSA nanoparticles	cRGD peptide	Nab technology	160 ± 23 nm	nanoparticles	GEM	BxPC-3 cell	The *in vitro* results confirmed thatcRGD- anchored nanoparticles can deliver gemcitabine to a pancreatic cancer cell line more efficiently
paclitaxel (PTX)-bound albumin nanoparticles	TRAIL	Nab technology	170~ 230 nm	nanoparticles	PTX and TRAIL protein	Mia Paca-2 cells	TRAIL/PTX HSA-NP would have potential as a novel apoptosis-based anticancer agent because of enhanced *in vitro* and *in vivo* performance.
Miscellaneous							
HSA nanoparticles	-	high pressure homogenizatio n	170.5 ± 4.0	nanoparticles	Cabazitaxel (CBX) and indocyanine green (ICG)	4T1, PC3, C6 cells	CBX and ICG could be co-delivered to the tumor tissue specifically viareceptor mediated pathway and possessed high accumulation in the tumor area
MTX–HSA conjugated	Biotin	MTX firstly conjugated	111.46 nm-	Albumin-drug	Methotrexate (MTX)	4T1 breast cancer cells	The *in vivo* anticancer experiment showed that biotin

nanoparticles		with HSA by using EDC and NHS and this conjugate is further cross-linked by EDC to form nanoparticles	144.36 nm	conjugated nanoparticles			targeted MTX-HSA NPs had stronger antitumor activity and lower toxic effect than non-targeted MTX-HSA NPs and free MTX in a mouse breast tumor model.
BSA nanoparticles	Folate	Desolvation	195.3 ± 5.6 nm,	nanoparticles	Bexarotene (BEX)	A549 and MCF-7 cells	Both the BEX-BSANPs and FA-BEX-BSANPs induced an enhanced cancer cell apoptotic effect in contrast to BEX solution.
Albumin nanoparticles	TRAIL	Modified desolvation and Electrostatic layer by layer assembly	Less than 200 nm	Core shell nanoparticles	DOX and TRAIL	H460 and DOX resistance L929 cells.	The assembled core/shell structure of the nanoparticles can be internalized more easily with the cancer cells, which attributes to TRAIL binding with death receptors.
BSA nanoparticles	Hyaluronic acid (HA)	Modified desolvation and Layer by layer assembly	119-238 nm	Core shell nanoparticles	DOX	MDA-MB-231 cells	Nanoparticles with HA as the final layer showed maximum cellular uptake in MDA-MB-231 cells owing to the CD44 receptor-mediated endocytosis and hence, exhibited more cytotoxicity as compared to free Dox.
Lipid Hybrid Albumin Nanoparticle	-	High pressure homogenization	128.4 ± 12.9 nm	Lipid Hybrid Albumin Nanoparticle	Pirarubicin (THP)	-	significantly reduced bone marrow suppression, cardiotoxicity, renal toxicity, and gastrointestinal toxicity

Targeted albumin based nanoparticles	Cyclic RGD peptide	Self-assembly	30 nm	Nano micelles	DOX	Human melanoma cells (M21+)	Higher uptake and longer retention of DOX with RGD modified micelles as compare to non modified micelles and free DOX	
Multi-stimuli-responsive biohybrid nanoparticles		Self-assembly	30-60 nm	Nano micelles	-	HepG2 cells	pH responsive, temperature responsive and enzyme responsive delivery. Highly biocompatible and higher internalization of nanoparticles by cells make it suitable candidate for cancer therapy	
Aluminium hydroxide/iron-Ce6-albumin nanoparticles (Al-Ce6-ANPs)	-		25.25 ± 2.1 nm; 33.4 ±4.55 nm	Nanoparticles	Ce6	B16F10; bone marrow derived dendritic cells (BMDCs); C57BL/6 mice	The results demonstrated efficient internalization of Ce6 in B16F10 cells with several endocytic pathways and Al-Ce6-ANPs found to be safe and biocompatible. Al-Ce6-ANPs were able to stimulate immune response which boosted the efficacy of phototherapy.	(30)
cRGD-BSA-PPy-PhENH2-NPs	cRGD-BSA		31.48 ± 5.62; 49.84 ±7.46 nm	nanoparticles		U87; Female BALB/c nude mice	In vivo study revealed that as compared to cRGD-BSA-PPy-PhENH2- NPs, BSA-PPy-PhENH2- NPs were efficiently accumulated at tumor region and lead to outstanding photothermal	(31)

							therapy of tumors.	
BSA-5BMF (small molecule) complex	-BMF		7.53 nm			U 87 M; MDA-MD-231; NIH-3T3 cells; Female Athymic nude mice	Both 5BMF alone and BSA-5BMF complex demonstrated toxicity towards tumor cells in the micro and nano-range amount	(32)

2.5.4 Clinically approved albumin nanocarriers and ongoing clinical trials

Owing to huge advancement in albumin based drug delivery systems, especially nanocarriers, the albumin based nano formulations have been projected as potential carrier systems feasible for market translations, leading to their approval for clinical trials. The first product to get green flag for clinical trials dates back to late nineties with the first FDA approval for human use in 2005 Abraxane developed by Celgene Ltd. Thereafter, various other albumin based formulations for cancer therapy and other diseases such as diabetes got approval for clinical trials, some of which made it to the market. Some of the major albumin based formulations specifically for cancer treatment and diagnosis have been summarized in Table 2.4. Vast amount of research is still going on in the same area and sooner or later we will see albumin based novel therapeutic and diagnostic platforms getting approval for cancer treatment which will serve as a major stepping stone for better healthcare system for cancer patient (25).

Table 2.4: Clinically approved albumin nanocarriers for therapy and diagnosis of cancer and other diseases and ongoing clinical trials for cancer therapy (modified from: Kudarha et al 2017)(25)

Name and company	Particle type/ drug	Approved application/ indication	Approval (year)	Investigated application/ indication	ClinicalTrials.gov identifier
Abraxane (Celgene)	Albumin-particle bound paclitaxel	Advanced non small cell lung cancer (surgery or radiation is not an option) Metastatic breast cancer (secondary), Metastatic pancreatic cancer (primary)	FDA (2005) EMA (2008)	Various cancers including: solid malignancies, breast, lymphomas, bladder, lung, pancreatic, head and neck, prostate, melanoma, or liver	295 studies mention Abraxane
Optison (GE Healthcare)	Human serum albumin stabilized perflutren microspheres	Ultrasound contrast agent	FDA (1997) EMA (1998)	Ultrasound enhancement for: lymph node, renal cell carcinoma, myocardial infarction, pulmonary transit times, or heart transplant rejections	11 currently active or recruiting studies
^{99m}Tc -Albures® (GIPHARMA)	^{99m}Tc aggregated albumin	Diagnostic agent used in screening of primary cancers		Various cancers including breast cancer, lymphomas, esophageal squamous cell carcinoma and other solid tumors	-
^{99m}Tc -Nanocoll® (GIPHARMA)	^{99m}Tc aggregated albumin	Diagnostic agent used in detection of metastasis		Various cancers including breast cancer, lymphomas, esophageal squamous cell carcinoma and other solid tumors	-
Levemir® (Novo Nordisk)	(Insulin detemer) Albumin binding derivative of human insulin	Diabetes type 1 and type 2	Europe (2004) FDA (2005)	Diabetes type 1 and type 2	-

Victoza® (Novo Nordisk)	(Liraglutide) Albumin binding derivative of GLP-1	Diabetes type 2	Europe (2009) FDA (2010)	Diabetes type 2	-
Eperzan®/Tanzeum® (Albiglutide) (Glaxo Smith Kline)	GLP-1 receptor agonist genetically fused with albumin	Diabetes type 2	FDA (2014)	Diabetes type 2	
ABI-008 (Celgene)	Albumin bound Docetaxel	-	-	Prostate and colon cancer	Phase I/II completed
ABI-009 (Aadi with Celgene)	Albumin bound rapamycin	-	-	Bladder cancer, PEComa, or pulmonary arterial hypertension	NCT02009332 (Phase I/II) NCT02587325 (Phase I) NCT02494570 (Phase II)
ABI-010 (Celgene)	HSP90 inhibitor	-	-	Cancer (hematological malignancies)	Withdraw before enrollment
ABI-011 (NantBioScience)	Albumin bound thiocolchicine analog (IDN 5405)	-	-	Solid tumors or lymphomas	NCT02582827 (Phase I)
Aldoxorubicin (INNO-206 or DOXO-EMCH) (CytRx, Inc.)	Albumin-Doxorubicin conjugate	-	-	Cancer (soft tissue sarcoma, small cell lung cancer etc.)	Phase I completed and Phase II ongoing for small cell lung cancer
MTX-HAS (Access Pharmaceuticals Inc.)	Albumin-drug conjugate	-	-	Cancer and autoimmune disease	Phase II
Ozoralizumab (Ablynx)	Albumin binding antibody derivative	-	-	Rheumatoid arthritis	Phase II completed
Albuferon®/Zalbin / Jouleferon (Human genome Sciences in	Genetically Fused protein of albumin and INFalpha-2b	-	-	Hepatitis C	Phase III completed, Development ceased

collaboration with Novartis)					

Table 2.5: List of albumin nanoparticles in recent clinical trials

Title	Sponsor	Collaborator /responsible party	Drug	Status	Phase	ClinicalTrials.gov identifier
Paclitaxel Albumin-Stabilized Nanoparticle Formulation and Bevacizumab in Treating Patients With Stage IV Melanoma That Cannot Be Removed by Surgery or Gynecological Cancers	Mayo clinic	National Cancer Institute (NCI)	Paclitaxel	Recruiting	I	NCT02020707
Expanded Access for ABI-009 in Patients With Advanced PEComa and Patients With a Malignancy With Relevant Genetic Mutations or mTOR Pathway Activation	Aadi LLC	Aadi LLC	ABI-009 (Sirolimus)	Available	-	NCT03817515
Nanoparticle Albumin-Bound Rapamycin, Temozolomide, and Irinotecan Hydrochloride in Treating Pediatric Patients With Recurrent or Refractory Solid Tumors	Children's Oncology Group	National Cancer Institute (NCI)	Temozolamide, Irinotecan Hydrochloride	Recruiting	I	NCT02975882
Paclitaxel Albumin-Stabilized Nanoparticle Formulation, Gemcitabine, and Bevacizumab in Treating Patients With Metastatic Breast Cancer	Alliance for Clinical Trials in Oncology	National Cancer Institute (NCI)	Gemcitabine, Bevacizumab, paclitaxel	Completed	II	NCT00662129
Nanoparticle Albumin-Bound Rapamycin and Pazopanib	University of Washington	National Cancer	Rapamycin, Pazopanib	Recruiting	I/II	NCT03660930

Hydrochloride in Treating Patients With Advanced Nonadipocytic Soft Tissue Sarcomas		Institute (NCI) Aadi, LLC	hydrochloride			
Albumin-Bound Paclitaxel Followed by Epirubicin in Combination With Cyclophosphamide in Triple Negative Breast Cancer	Fudan University	Zhimin Shao, Fudan University	Paclitaxel	Recruiting	IV	NCT03799679
Albumin-Bound Paclitaxel Combined With Carboplatin as Neoadjuvant Chemotherapy in Luminal B/HER-2 Negative Breast Cancer	Fudan University	Zhimin Shao, Fudan University	Paclitaxel	Recruiting	IV	NCT03799692
Weekly Nanoparticle Albumin-Bound Paclitaxel (Abraxane) + Weekly Cetuximab + Radiation Therapy (IMRT, Intensity-Modulated Radiation Therapy) in Patients With Stage III-IVB Head and Neck Squamous Cell Carcinoma (HNSCC)	Memorial Sloan Kettering Cancer Center	Celgene Corporation National Comprehensive Cancer Network	Cetuximab, Paclitaxel	Completed	I	NCT00736619
Nanoparticle Albumin-Bound Rapamycin in Treating Patients With Advanced Cancer With mTOR Mutations	Mayo Clinic	National Cancer Institute (NCI)	Rapamycin	Completed	Early phase I	NCT02646319
Intraperitoneal Paclitaxel Albumin-Stabilized Nanoparticle Formulation in Treating Patients With Advanced Cancer of the Peritoneal Cavity	City of Hope Medical Center	National Cancer Institute (NCI) National Comprehensive Cancer Network	Paclitaxel	Completed	I	NCT00825201
Paclitaxel Albumin-Stabilized Nanoparticle Formulation in Treating	University of Washington	National Cancer	Paclitaxel	Active, not recruiting	II	NCT01620190

Patients With Previously Treated Advanced Non-small Cell Lung Cancer		Institute (NCI) Celgene Corporation				
Paclitaxel Albumin-Stabilized Nanoparticle Formulation in Treating Patients With Recurrent or Persistent Ovarian Epithelial Cancer, Fallopian Tube Cancer, or Primary Peritoneal Cancer	Gynecologic Oncology Group	National Cancer Institute (NCI)	Paclitaxel	Completed	II	NCT00499252
Treatment of Triple-negative Breast Cancer With Albumin-bound Paclitaxel as Neoadjuvant Therapy: a Prospective RCT	Shengjing Hospital	Caigang Liu, Shengjing Hospital	Paclitaxel + Carboplatin	Not yet recruiting	III	NCT04137653
Paclitaxel (Albumin-bound) and Oxaliplatin for Advanced Hepatobiliary and Malignant Tumors	Dong Wang	Dong Wang, Third Military Medical University	Paclitaxel + Oxaliplatin	Not yet recruiting	II/ III	NCT04060472
A Phase II Trial of Abraxane™ Given Weekly as a Single Agent in First-line Treatment of Metastatic Breast Cancer	Veeda Oncology	Celgene Corporation	Paclitaxel	Completed	II	NCT00251472

2.6 Surface functionalization of Albumin nanoparticles for targeted drug delivery

Surface functionalization of albumin nanoparticles is required to modulate properties of albumin nanoparticles surface and improve the delivery system's site specific localization. Albumin possesses several binding sites and functional groups viz. amino and carboxyl groups provide many opportunities for other materials to be functionalized over the surface of albumin. Surface functionalization can be achieved by two distinct techniques viz. covalent and non-covalent bonding. The covalent bonding includes the two different groups of distinct material for example amino group of albumin and carboxyl group of another material of interest (carbodiimide chemistry), when SH- group is involved then the process is known as Michael addition)(28,33). The non-covalent technique involves the electrostatic interaction between surface of NPs and material of interest to be attached. In case of surface modified albumin NPs, usually but not limited to albumin act as carrier for delivery drugs, while ligand is utilized to modulate circulation half-life, stability and pharmacokinetic parameters along with release pattern of drug and targeting efficiency. There are several strategies viz. disulphide bridging, carbodiimide chemistry, Michael addition and biotin/avidin ligation (34).

2.6.1 Hyaluronic acid (HA) as targeting ligand

HA is natural, anionic, non-sulfated glycosaminoglycan that consists of β-1,4 linked D-glucuronyl-β-(1,3) (Gln)-N-acetyl-D-glucosamine and are widely distributed throughout epithelial, neural and connective tissues (35). HA is the largest polysaccharide in the body, with an average molecular weight of 1-8 MDa(36,37). Human skin also contains large amount of HA i.e. 400-500 µg HA/g (38). In other organs, the content of HA can vary from approximately from 1 to 100 µg HA/g (39). HA plays significant role in various biological processes, cancer metastasis, cell migration, cell differentiation and wound healing (40). Additionally, CD44, a glycoprotein, is HA receptor and are overexpressed in large number of mammalian cells and its interaction with HA is crucial for the growth and metastasis of cancer cells. A lot of attention has been attracted by the researchers towards investigation of HA as a targeting moiety in cancer therapy and cancer imaging. Recently, Shen et al. developed HA-functionalized erlotinib loaded BSA NPs using nanoprecipitation method for lung cancer treatment. The results demonstrated that NPs were spherical in shape with particle size in the range of 112 nm and greater drug loading and entrapment efficiency of 5.6% and 81.2%, respectively. The release study confirmed the sustained release of erlotinib with no burst release and it may be due to coating of HA over the surface of BSA NPs. The results further quoted that owing to the presence of HA the cellular uptake efficiency of BSA NPs was enhanced in A549 cells. Furthermore, the results also showed that there was no significant difference in the pharmacokinetic parameters between HA-BSA NPs and BSA NPs, when administered intravenously. However, in-vivo antitumor activity revealed that HA-erlotinib-BSA NPs were able to significantly supress tumor growth and no relapse was observed after 30 days of treatment(41). Edelman et al. fabricated HA conjugated

PTX and imidazoacridinones loaded BSA NPs. The results demonstrated that HA-BSA NPs with particle size of 15 nm were able to internalize by CD44-receptor mediated endocytosis. The results further confirmed that HA-BSA NPs were highly internalized in CD44 overexpressing ovarian cancer cells whereas not in CD44 lacking cells. The results showed that HA-BSA NPs showed significant reduction in tumor overexpressing CD44 receptors(42). Thus it can be concluded that HA is potential targeting moiety for efficient site specific delivery or localization of nanocarriers. Some of the applications of HA as a targeting ligand is summarized in table 2.6.

Table 2.6 Application of HA as targeting ligand

Architecture	**Surface functionalization**	**Drug**	**Particle size (nm)**	**In-vitro/ In-vivo modalities**	**Applications**	**Ref.**
Lf/HA@Lignosulfonate NPs	Lf/HA	Quinacrine	138	PANC-1 cells, Adult male Balb/c mice	Dual targeting efficiency was increased (3-folds decrease in IC50) as compared to individual NPs. Enhanced the ability to inhibit migration and invasion of pancreatic cancer cells.Significantly reduced tumor volume by dual-targeting strategy.	(43)
HA-BSA NPs	HA	DOX	119-238	MDA-MB-231 cells	Nanoparticles with HA asthe final layer showedmaximum cellular uptakein MDA-MB-231 cellsowing to the CD44receptor-mediatedendocytosis and hence,exhibited morecytotoxicity as comparedto free Dox.	(44)
HA-HSA NPs	HA	Erlotinib	112.5	A549 cells; SD rats	The study revealed high efficiency uptake of HA-HSA NPs in A549 cells. In-vivo study showed that despite of showing higher bioavailability by HA-HSA NPs, there were no significant difference in the pharmacokinetic parameters between HA-HSA NPs and HSA-NPs.	(41)
HA-BSA-NPs	HA	PTX	15	A2780 and SKOV3	The results showed that HA-BSA NPs were efficiently internalized in CD44 receptor containing cells	(42)

				cells	as compared to the cells in which CD44 receptors are absent.In similar manner, the highest toxicity was exhibited to the cells containing CD44 receptors due to selective targeting of HA. The results also confirmed that CD44 blocking antibody when exposed to cells, the HA-BSA NPs internalization was diminished, hence CD44 receptors play significant role when used HA as targeting moiety.	
HA-BSA NPs	HA	All-trans-retinoic acid (ATRA)	180.63	B16F10 cells	The study revealed that HA-BSA NPs were efficiently taken up by B16F10 cells (CD44 receptors-enriched cells) as compared to unmodified NPs In-vivo imaging revealed greater accumulation of HA-BSA NPs in tumor-bearing lung of mouse.	(45)

2.6.2 Chondroitin sulphate (CS) as targeting ligand

CS is form of glycosaminoglycans and composed of repeated units of disaccharides i.e. b-1,4-linked D-glucuronic acid (GlcA) and b-1,3-linked N-acetyl galactosamine (GalNAc) including sulphated groups at distinct positions of CS. There are several types of CS viz. chondroitin 4,6-sulphate, chondroitin 2,6-sulphate, chondroitin 6-sulphate and chondroitin 4-sulphate, depending on position of sulphate group in CS(46). It has been reported that CS found in animal tissues with miolecular weight of 20 KDa which denotes over 100 single sugar units along with sulphated groups. Squid cartilage is rich source of chondroitin 4,6-sulphate; Shark cartilage is rich source of chondroitin 6-sulphate and porcine and bovine cartilage are rich source of chondroitin 4-sulphate. CS is naturally occurring anionic mucopolysaccharide and most abundantly in blood vessels, skin, bone, nerve tissue, ECM and cartilages of mammals (46,47). CS has attracted lot of attention and investigation for its utilization in gene and drug delivery owing to its biodegradable and biocompatible nature. It can be easily lipophilically modified owing to the presence of several functional groups such as hydroxyl and carbolic groups on molecular chain. For instance, Liu et al. synthesized CS-DOX conjugation and was then embedded in PLGA NPs. The results revealed that CS-DOX PLGA NPs were efficiently passed through peripheral tumor barrier and into tumor cell nucleus taking the benefit of CD44 cell-mediated endocytosis and enhanced permeability. In brief, the results showed that strong DOX

fluorescence was detected in U251 cells especially in the nucleus when exposed with CS-DOX PLGA as compared to free DOX. It may be due to free DOX may be internalized into cells with passive diffusion whereas CD44-mediated endocytosis pathway was followed by CS-DOX PLGA owing to the presence of CS in the composite. It was further confirmed by CD44 blocking assay which revealed that before blocking CD44 receptors the CS-DOX PLGA and CS-DOX displayed higher intensity of red fluorescence, whereas, when CD44 receptors were blocked then it displayed low intensity of red fluorescence, indicating the involvement of CD44 receptors in the efficient internalization of NPs(48). Additionally, with the hypothesis that CS can accumulate in golgi apparatus which shows significant role in signalling pathway for metastasis, Li et al. synthesized retinoic acid (RA) conjugated CS (RA-CS). The results demonstrated that RA-CS were efficiently accumulated in the golgi apparatus and at acidic microenvironment prompt the RA release. Further study confirmed that RA-CS were able to efficiently inhibited the multiple metastasis-associated proteins expression by disrupting structure of golgi apparatus. Further. PTX was loaded in RA-CS for synergistic action. The results demonstrated that addition of PTX in RA-CS showed inhibited in-vitro migration, invasion and angiogenesis and suppressed tumor growth and metastasis in 4T1-Luc bearing mice(49). Furthermore, Lee et al. developed PEG functionalized DOX loaded CS A-deoxycholic acid NPs (PEG-CS-DOX-D-NPs) for ovarian cancer. The DOX showed sustained and pH-responsive drug release from PEG-CS-DOX-D-NPs. The cellular internalization was by CD44-mediated endocytosis pathway with CD44 receptor positive ovarian cells (SKOV-3 cells). The results also revealed that non-PEGylated NPs were responsible for greater drug clearance as compared to PEGylated NPs and was confirmed by pharmacokinetics after intravenous administration(50). Some applications of CS as targeting ligand is summarized in table 2.7.

Table 2.7 Application of CS as targeting ligand

Architecture	Surface function alization	Drug	Particl e size (nm)	In-vitro/ In-vivo modalities	Applications	Ref.
CS-PEG-PLGA NPs	CS	5-FU	100 - 200	MCF-7; MDA-MD-231 cells	Prepared NPs were able to sustained the release of 5-FU till 48 h, whereas, the unomodified NPs released drug within 24 h. As compared to free drug, the CS-PEG-PLGA NPs were less haemolytic CS-PEG-PLGA NPs were responsible for providing efficient cytotoxicity to both the cell line as compared to free drug.	(51)

Chitosan-CS-NPs and Chitosan-CS-lecithin NPs	Chitosan	Curcumin	130	MCF-7 cells	The study confirmed that NPs showed two-phase process for release i.e. diffusion controlled dissolution and release of curcumin controlled by dissolution of the polymer. Both types of NPs were able to exhibit efficient cytotoxicity to MCF-7 cells as compared to free drug.	(52)
PEG-CS-A-deoxycholic acid (D) NPs	PEG	DOX	247	SKOV-3 cells	Flow cytometry and CLSM study confirmed the internalization of NPs and demonstrated that NPs were internalized by CD44-mediated pathway	(50)

2.6.3 Lactoferrin (Lf) as targeting ligand

Lf is a glycoprotein belongs to transferrin family consists of about 690 amino acid containing polypeptide chain. These amino acids are folded into two globular lobe and each of contains one iron-binding site. Lf shows 60-80% identical sequence with transferrin (Tf). Lf is one of the most widely used tumor targeted moiety owing to the presence of Lf receptors in overexpressed manner in most of the cancer cells(53). Additionally, Lf receptors are not only present on cancer cells but also present on BBB resulting into Lf transport across BBB in-vivo and in-vitro receptor-mediated transcytosis. As compared to Tf, Lf has less plasma concentration under physiological conditions which makes Lf an efficient target ligand for tumors(54). For instance, Sharifi et al. developed two different iron-based NPs viz. magnetite and maghemite NPs and both were coated with Lf. Lf was conjugated over both the NPs using carbodiimide chemistry. The results revealed that Lf-magnetite NPs exhibited highest cytotoxicity to 4T1 cells as compared to non-Lf coated NPs, however, the cytotoxicity of all the samples were concentration dependent. The ROS generation study demonstrated that magnetite, maghemite, Lf-magnetite and Lf-maghemite showed 169, 121, 207 and 171 unit DCF intensity respectively relative to control group. The overall results concluded that based on ROS generation, the Lf coated NPs were responsible for increasing the mortality of 4T1 cells(55). Additionally, Kumari et al. fabricated TMZ loaded Lf-NPs (TMZ-Lf-NPs) in glioma treatment. The results indicated that prepared TMZ-Lf-NPs were efficiently penetrated through BBB using transcytosis mediated pathway and also preferentially tumor cell uptake. The release study demonstrated that TMZ was released in response to acidic microenvironment of tumor. In-vivo study confirmed the

significant reduction in tumor volume, higher tumor cell apoptosis and improved median survival in glioma bearing mice(56). Recently, Ali et al. synthesized Lf conjugated pemetrexed and ellagic acid co-loaded silica NPs (SiNPs) (Lf-PMX-EA-SiNPs) for synergistic activity in breast cancer treatment. In this, EA was physically adsorbed within the pores of Si and PMX was conjugated with Lf using carbodiimide chemistry. The release study demonstrated that Lf-PMX-EA-SiNPs showed sequential faster EA release followed by PMX sustain release. As compared to individual free drug, the combination of drug in carrier exhibited highest cytotoxicity to MCF-7 breast cancer cells. The cellular uptake was found to be from Lf-mediated endocytosis(57). Now-a-days most of the conventional therapies are exhibiting weak potential towards cancer treatment because of metastasis, drug resistance and toxicity limit. Keeping in mind, Sharifi et al. developed a multi-modal Lf conjugated DOX loaded mesoporousmaghemite NPs (Lf-DOX-MMNPs) for targeted delivery, photothermal therapy/chemotherapy and magnetic field in breast cancer treatment. The results revealed that this carrier was found to be efficient as multi-modality platform(58). Some applications of LF as targeting ligand is summarized in table 2.8.

Table 2.8: Application of Lf as targeting ligand

Architecture	**Surface functionalization**	**Drug**	**Particle size (nm)**	**In-vitro/ In-vivo modalities**	**Applications**	**Ref.**
Lf-SPIONs	Lf	-	75	HEK 293 cells; HL-7702; ECV 304 cells; rat model of Ce6 glioma	Lf-SPIONs exhibited more sensitivity and better picture to depict brain glioma on MR image as compared to plain SPIONs Till 48 h of i.v. injection, Lf-SPIONs exhibited significant T2-weighted images of brain glioma	(59)
Lf/HA@ Lignosulfonate NPs	Lf/HA	Quinacrine	138	PANC-1 cells, Adult male Balb/c mice	pH-responsive drug release was obtained dual targeting efficiency was increased (3-folds decrease in IC50) as compared to individual NPs Enhanced the ability to inhibit migration and invasion of pancreatic cancer cells. Significantly reduced tumor volume by dual-targeting strategy.	(43)
Lf-SiNPs	Lf	Pemetrexed and	284	MCF-7 cells;	Release study demonstrated that Ellagic acid was released faster followed by sustained release of	(57)

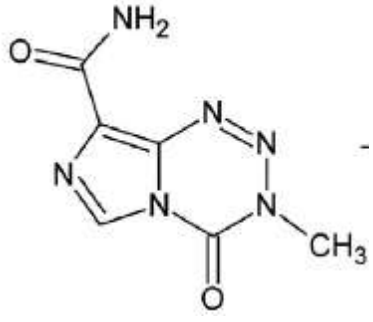
		Ellagic acid			pemetrexed As compared to free drug, Lf-SiNPs exhibited greater cytotoxicity to MCF-7 breast cancer cells. Lf-SiNPs followed Lf-mediated endocytic pathway in MCF-7 cells3	
Lf-IONPs	Lf		14-26	Ce6 glioma cells	The results reported that as compared to plain IONPs, with increased in particle of Lf-IONPs improved magnetic particle spectrometry signal intensity and spatial resolution. Lf-IONPs showed significant cellular uptake of 5-fold increase in MPS signal as compared to without Lf coating	(60)
PEG grafted BSA NPs	Lf	DOX	150-155	BCECs and C6	Better permeationenhancement property,increased dual targetingeffect of nanoparticlesfor brain delivery andfacilitated the uptake ofDOX in the brain tissue	(61)
Lf-LA/GA-oily core nanocapsules	Lf and LA/GA	Sorafenib and quercetin	92.64 – 230.2	HepG2 cells; Hepatocarcinoma cancer cells bearing mice	Dual targeting NC displayed higher cellular internalization in HepG2 cells as compared to individual targeting of NCs. After 48 h, duar targeting NC showed 2-fold reduction in half-maximum inhibitory concentration as compared to free combination therapy. In-vivo study demonstrated that dual targeting NCs showed efficient downregulation of mRNA expression level of NF-α and NFk-B as well as suppression of Ki-67 protein expression level.	(62)
Curcumin-Lf nanostructure	-	Curcumin	165	HCT116	The results revealed that Curcumin-Lf nanostructure were able to show higher toxicity and cellular uptake in HCT116 cells as compared to free curcumin.	(63)
Lf-PEG-PLA NPs	Lf	Coumarin 6	109	bEnd.3 cells; KM mice	Lf-PEG-PLA NPs showed higher cellular uptake as compared to unmodified NPs In-vivo demonstrated that Lf-PEG-PLA NPs were able to increase	(54)

					concentration of coumarin 6 in brain 3-folds higher as compared to unmodified NPs after intravenous administration.	

2.7 Temozolomide

Temozolomide (TMZ) is, first synthesized in 1984, a monofunctional alkylating agent of triazene class and now-a-days used as first line drug for the treatment of glioblastoma. It acts by inducing alkylation of DNA and crosslinking that leads to DNA damage and subsequent cell death. This is used in conditions where surgery or radiation therapy are ineffective or not possible, or in combination therapy with surgery or Radiation therapy as initial treatment. This drug is used to attenuate the symptoms or for prolonging the survival of the patient as it is not successfully curative. However, TMZ can induce systemic toxicity including thrombocytopenia, lymphopenia, and myelodysplasia. It was reported that 7% of patients had to discontinue the treatment due to the presentation of these toxic side effects (64).

2.7.1 Drug profile of TMZ (65)

Properties	**Description**
Chemical IUPAC name	3-methyl-4-oxo-3,4-dihydroimidazo(5,1-d)(1,2,3,5)tetrazine-8-carboxamide
Proprietary name	Temodar and Temodal
Empirical formula	$C_6H_6N_6O_2$
Molecular weight	194.1508g/mol
Structure	
Melting point	212^0C
Solubility	Water Solubility is 5.09 mg/ml.
pKa	10.51(Strongest Acidic)/ -3.6(Strongest Basic)

log P	-1
BCS Class	
Category	Alkylating agent
Mechanism of action	Temozolomide is not active until it is converted at physiologic pH to MTIC. It is suggested that MTIC then alkylates DNA at the N7 position of guanine, O3 position of adenosine, and O6 position of guanosine, with the most common site being the N7 position. This methylation of guanine residues lead to single and double-strand DNA breaks and subsequent apoptotic cell death. It is suggested that the N7-methylguanine plays a critical role in the antitumor activity of the drug, as there is a correlation between the sensitivity of tumor cell lines to temozolomide and the activity of O6-alkylguanine alkyltransferase, which is the DNA repair protein that specifically removes alkyl groups at the O6 position of guanine. Cells lines that have lower levels of AGT are more sensitive to the cytotoxicity of temozolomide. It is also suggested that cytotoxic mechanism of temozolomide is related to the failure of the DNA MMR system to find a complementary base for methylated guanine. The DNA MMR system is involved in the formation of a number of proteins that remove methylated guanine. Evidence shows that when this repair process is targeted to the DNA strand opposite the O6-methylguanine, its inability to find the correct target leads to long-lived nicks in the DNA. The accumulation of these nicks lead to the inhibition of replication in the daughter cells, thereby blocking the cell cycle at the G2-M boundary.
Indications	For the treatment of adult patients diagnosed with anaplastic astrocytoma whose disease has progressed after therapy with nitrosourea and procarbazine, as well as concomitantly with radiation therapy for treatment of newly diagnosed

	glioblastomamultiforme. Also used as maintenance therapy for glioblastomamultiforme.
Adverse reactions	The most common side effects with temozolomide are nausea, vomiting, constipation, loss of appetite, alopecia, headache, fatigue, convulsions, rash, neutropenia or lymphopenia, and thrombocytopenia. Temozolomide is genotoxic, teratogenic and fetotoxic and should not be used during pregnancy. Lactating women should discontinue nursing while receiving the drug because of the risk of secretion into breast milk.
Contraindications	Temozolomide is contraindicated in people with hypersensitivity to it or to the similar drug dacarbazine. The use of temozolomide is not recommended in people with severe myelosuppression
Drug interactions	Warfarin, abciximab and 4-hydroxycoumarin when individually combined with Temozolamide may increase high risk of bleeding. Temozolamide when combined with Abacavir, decreases the excretion rate of Abacavir, leading to its higher serum level
Absorption	Rapid and complete absorption in the gastrointestinal tract
Distribution	0.4 L/kg
Metabolism and Excretion	About 38% of the administered temozolomide total radioactive dose is recovered over 7 days: 37.7% in urine and 0.8% in feces.
Marketed products	Only one dosage form is available and it's for oral route. **Brand name**: Temodal and Act Temozolamide It is available in 5 mg, 140 mg, 255 mg capsules for oral administration
Dose and dosing frequency	**Anaplastic Astrocytoma** **Initial:** 150 mg/m² PO/IV qDay for 5 days; repeat at 28-day cycles

	Maintenance: May increase/maintain dose at 200 mg/m² PO/IV qDay for 5 days/28-day cycle if ANC >1500 mm³ and platelets >100,000 mm³ Infuse IV over 90 minutes **Glioblastoma Multiforme** **Initial:** 75 mg/m² PO/IV qDay for 42 days concomitant with focal radiotherapy Infuse IV over 90 minutes **Continue treatment** Continue for up to 49 days if the criteria listed below are met ANC ≥1,500/mm³, Platelet >100,000/mm³; CTC Grade 1 or lower

2.7.2 Schematic representation of mechanism of action of TMZ

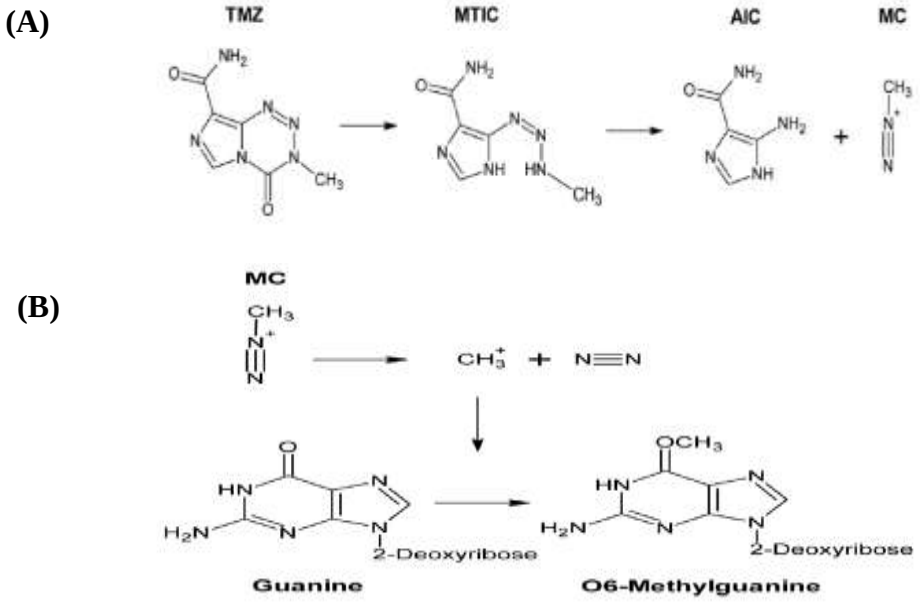


Figure 2.9: (A) Activation of TMZ in active metabolite methyl diazonium cation (MC) and (B) mechanism of DNA alkylation

2.7.3 Challenges and limitations of TMZ

TMZ, due to its less invasive administration, exhibits several advantages over both neurosurgical resection of the tumor and radiation therapy. Although TMZ has ability to cross blood brain barrier (BBB), it needs high systemic dose to reach therapeutic concentration in brain because of its short half-life. Its efficiency is also limited due to its nonspecific toxicity causing harmful side effects in healthy tissues. The previous studies proposed that continuous TMZ administration results into reduction in Nadir's platelet count. Furthermore, the efficacy of TMZ is vulnerable byseveral resistance mechanisms and biological barriers, as previously mentioned(64).It is hypothesized, therefore, that with the help of nanoparticles, TMZ can sustain its release which will control its pharmacokinetic parameters and hence avoid repeated administration. Due to this, various systemic side effects like oral ulceration, bone marrow suppression, fatigue, vomiting, nausea and headache are associated with TMZ therapy (64,66)**.** To overcome these side effects and to improve the therapeutic activity of TMZ via targeting TMZ in brain, novel drug delivery system is required.

2.7.4 Nanocarriers for delivery of TMZ

TMZ's low bioavailability in the brain tissue, high toxicity, and cell resistance continue to be highlighted as major challenges in developing formulations for its clinical use. Thus, several studies using nanocarriers for TMZ delivery have been reported. Some of these studies are summarized in Table 2.9

Table 2.9 Currently Developed Nanocarriers for TMZ Delivery

Architecture	Targeting Ligand	Size (nm)	In vitro/ in vivo model	Findings	Ref.
TMZ-PLGA NPs	n.a.	150	uptake studies, Cytotoxicity	PLGA nanoparticles caused a sustained release of the drug and showed a higher cellular uptake. The drugformulations also affected the cellular proliferation and motility.	(67)
TMZ-PLGA NPs	n.a.	200	Cytotoxicity studies	PLGA NPs are not suitable carrier for TMZ as poor loading and encapsulation efficiency obtained and also high IC_{50} values of glioblastoma cells to TMZ was obtained.	(68)
TMZ-PLGA NPs	Tf	~120	Cytotoxicity study	Enhanced uptake and brain loacalization of Lf appended nanoparticles.	(69)

TMZ-PLGA NPs	Folate	400-600	n.a.	Slow release of TMZ from nanoparticles	(70)
Co-delivery of TMZ and Paclitaxel via PLGA NPs	n.a.	200	Cytotoxicity studies, Biodistribution and tumor growth studies with mice	PTX/TMZ-NPs showed better inhibition effect to U87 and C6 cells than single drug NPs. Also showed best anti tumor activity.	(71)
Co-delivery of TMZ and Dox via chitosan grafted PLA NPs	n.a.	150-350	n.a.	The continuous drug release without an initial burst in different physiological media was obtained.	(72)
Co-delivery of TMZ and 5-FU via Chitosan NPs	n.a.	100-200	n.a.	Results indicated effect of weight ratio of polysaccharides and pH of media on drug loading and release rate.	(73)
Co-delivery of TMZ, micro RNA and gold NPs via Chitosan nanogels	Folate	100	Cytotoxicity, tumor growth studies in mice	Enhanced cellular uptake and antitumor efficacy	(74)
TMZ-Poly(β-L-malic acid) NPs	mAb-totransferrin receptor and trileucine	15	Cytotoxicity and uptake	Uptake was receptor mediated endocytosis driven and significant reduction in cell viability	(75)
TMZ-Chitosan NPs	Chlorotoxin	50	Cytotoxicity, uptake and biodistribution in mice	Enhanced stability in physiological pH, half life and cellular uptake	(76)
TMZ-Liposome	Anti-Tf receptor antibody	40	Cytotoxicity, uptake studies Biodistribution, survival and tumor growth studies with mice	Significantly prolonged survival in mice bearing intracranial GBM tumors along with improved efficacy compared to standard TMZ	(77)

Co-delivery of TMZ and quercetin Liposome	n.a.	100-300	Cytotoxicity, uptake studies Biodistribution, studies with rats	Liposomes showed enhanced efficacy in the U87 cells and U87/TR cells along with higher brain accumulation as indicated by in vivo studies.	(78)
Co-delivery of TMZ and Vincristine via SLNs and NLCs	n.a.	120	Cytotoxicity studies Biodistribution and tumor growth studies with mice	NLCs can delivered vincristine and TMZ more efficiently than SLNs	(79)
TMZ-NLCs	RGD	120	Cytotoxicity, uptake studies Biodistribution and tumor growth studies with mice	The U87MG cells were successfully inhibited by RGD-TMZ/NLCs in vitro along with the highest antitumor efficacy in vivo than the other formulation.	(80)
Co-delivery of TMZ and gene via NLCs	n.a.	179	Cytotoxicity, in-vivo gene transfection and anti tumor efficacy	Enhanced antitumor and gene transfection efficacy.	(81)
Co-delivery of TMZ and bromodomain inhibitor via Tf functionalized nanoparticles (Tf-NPs)	Tf		BBB passage in mice, Intracranial orthotropic model of GBM in mice	Increased DNA damage and apoptosis that correlates with a 1.5- to 2-fold decrease in tumor burden and corresponding increase in survival compared to equivalent free-drug dosing.	(82)
PLGA NPs	Anti-EPHA3-modified	145.9	Uptake, distribution and tumor growth inhibition study	In vivo imaging and distribution studies on the glioma-bearing rats showed that anti-EPHA3-modified NPs exhibited high fluorescence intensity in the brain and effectively accumulated to glioma tissues along with significantly higher tumor cell	(83)

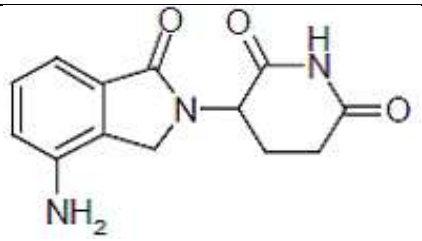
				apoptosis ($p < .01$) than that observed with other formulations and prolonged the median survival time of glioma-bearing rats to 26 days, which was 1.37-fold longer than that of PLGA NPs.	
TMZ and ICG loaded Iron oxide NPs	-		Apoptosis study on U87MG cells, western blot and RT-PCR	Fe_3O_4‐TMZ‐ICG MNPs with NIR laser irradiation lead to significantly enhanced anticancer effects on U‐87 MG glioblastoma cells through the modulation of intrinsic and extrinsic apoptosis genes, including Bcl‐2‐associated X protein, Bcl‐2, cytochrome c, caspase‐3, Fas associated via death domain and caspase‐8.	(84)
Multifunctional TMZ-loaded lipid superparamagnetic nanovectors	Streptavidin, Biotin, Anti-TfR-Abs	36	BBB model, Antiproliferati-ve and pro-apoptotic study on 3D models of GBM	Hybrid NPs were able to overcome theblood–brain barrier and to induce powerful anti-cancer effects on in vitro complex models	(85)

2.8 Lenalidomide (LND)

Lenalidomide (LND) was proposed for the treatment of multiple myeloma, for which thalidomide is an approved medicine and LND shares structural similarities with thalidomide. It is blockbuster drug and is an immunomodulatory agent with anti-tumor and antiangiogenic properties.

2.8.1 Drug profile (86)

Properties	**Description**
Chemical IUPAC name	3-(4-amino-1-oxo 1,3-dihydro-2*H*-isoindol-2-yl) piperidine-2,6-dione
Proprietary name	Revlimid
Empirical formula	$C_{13}H_{13}N_3O_3$

Molecular weight	259.3 g/mol
Structure	
Melting point	268.1 - 270.1 °C
Solubility	Water Solubility is 2.33 mg/ml. Soluble in organic solvent/water mixtures, and buffered aqueous solvents. Lenalidomide is more soluble in organic solvents and low pH solutions.
pKa	11.61(Strongest Acidic)/2.31(Strongest Basic)
log P	0.43
BCS Class	III
Category	Lenalidomide is the Immunomodulatory drug that is used to treat certain cancers like Multiple Myeloma (MM), Mantle Cell Lymphoma (MCL). It works by slowing or stopping the growth of cancer cells. It is also used to treat anemia in patients with certain blood/bone marrow disorders like Myelodysplastic Syndromes (MDS).Lenalidomide may lessen the need for blood transfusions
Mechanism of action	The mechanism of action of Lenalidomide remains to be fully characterized. Lenalidomide possesses immunomodulatory and antiangiogenic properties (Figure 2.2). Lenalidomide inhibits the secretion of pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and IL-12 and increases the secretion of anti-inflammatoryIl-10 cytokines from peripheral blood mononuclear cells. Lenalidomide inhibits the proliferation of various hematopoietic tumor cell lines, particularly the multiple myeloma. It enhances foetal hemoglobin expression upon $CD34^{+}$erythroid stem cell differentiation. Lenalidomide inhibits the expression of cyclooxygenase-2 (COX-2) but not COX-1 *in vitro*. It inhibits processes of angiogenesis including endothelial cell migration

	and tube formation. In addition to this, it inhibits the growth of hematopoietic tumor cells and, by inhibiting angiogenesis, reduces the growth of solid tumors *in vivo*. The molecular target of Lenalidomide is not known
Indications	Lenalidomide is indicated for the treatment of patients with Multiple myeloma (MM) in combination with Dexamethasone as maintenance following autologous hematopoietic stem cell transplantation (auto-HSCT).For the treatment of transfusion-dependent anemia due to low- or intermediate-1-risk Myelodysplastic syndromes (MDS) associated with a deletion 5q abnormality with or without additional cytogenetic abnormalities. In the patients with Mantle cell lymphoma (MCL) whose disease has relapsed or progressed after two prior therapies, one of which included Bortezomib
Adverse reactions	Most common adverse reactions include diarrhea, fatigue, anemia, constipation, neutropenia, thrombocytopenia, leucopenia, peripheral edema, insomnia, muscle cramp/spasms, abdominal pain, back pain, nausea, asthenia, pyrexia, upper respiratory tract infection, bronchitis, nasopharyngitis, gastroenteritis, cough, rash, dyspnoea, dizziness, decreased appetite, thrombocytopenia, and tremor
Contraindications	Pregnancy: Category X In the patients who demonstrated hypersensitivity to Lenalidomide.
Drug interactions	Digoxin: Periodic monitoring of digoxin plasma levels is recommended due to increased C_{max} and AUC with concomitant Lenalidomide therapy. Patients taking concomitant therapies such as erythropoietin stimulating agents or estrogen containing therapies may have an increased risk of thrombosis
Absorption	Lenalidomide, in healthy volunteers, is rapidly absorbed following oral administration with maximum plasma concentrations occurring

	between 0.625 and 1.5 hours post-dose. Co-administration with food does not alter the extent of absorption (AUC) but does reduce the maximal plasma concentration (C_{max}) by 36%. The pharmacokinetic disposition of Lenalidomide is linear. Cmax and AUC increase proportionately with increases in dose. Multiple dosing at the recommended dose-regimen does not result in drug accumulation. Oral bioavailability of Lenalidomide is <33%
Distribution	*In vitro* (^{14}C)-lenalidomide binding to plasma proteins is approximately 30%.
Metabolism and Excretion	Drug undergoes limited metabolism. Two identified metabolites are hydroxy-lenalidomide and N-acetyl-lenalidomide; each constitutes less than 5% of parent levels in circulation. The cytochrome P450 enzyme system is not involved with the metabolism of lenalidomide. In healthy volunteers, approximately two-thirds of Lenalidomide is eliminated unchanged through urinary excretion. The process exceeds the glomerular filtration rate and therefore is partially or entirely active. Half-life of elimination is approximately 3 hours
Marketed products	Only one marketed product is available. **Brand name**: REVLIMID It is available in 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg and 25 mg capsules for oral administration
Drawbacks of Recent therapies	Lenalidomide suffers from atypical biodistribution; hence need to be delivered precisely at site of action. It causes side effects like Warm Auto-Immune HemolyticAnemia (AIHA) when given via intravenous route and Bile acid Malabsorption Induced Diarrhea (BMID) when given via oral route
Dose and dosing frequency	a. **MM combination therapy**: 25 mg once daily orally on Days 1-21 of repeated 28-day cycle in combination with Dexamethasone.

	b. MM maintenance therapy: 10 mg once daily continuously on Days 1-28 of repeated 28-day cycles. **c. MDS**: 10 mg once daily. **d. MCL**: 25 mg once daily orally on Days 1-21 of repeated 28-day cycles. In case of renal impairment the starting dose can be adjusted based on the creatinine clearance value

2.8.2 Schematic representation of mechanism of action of LND

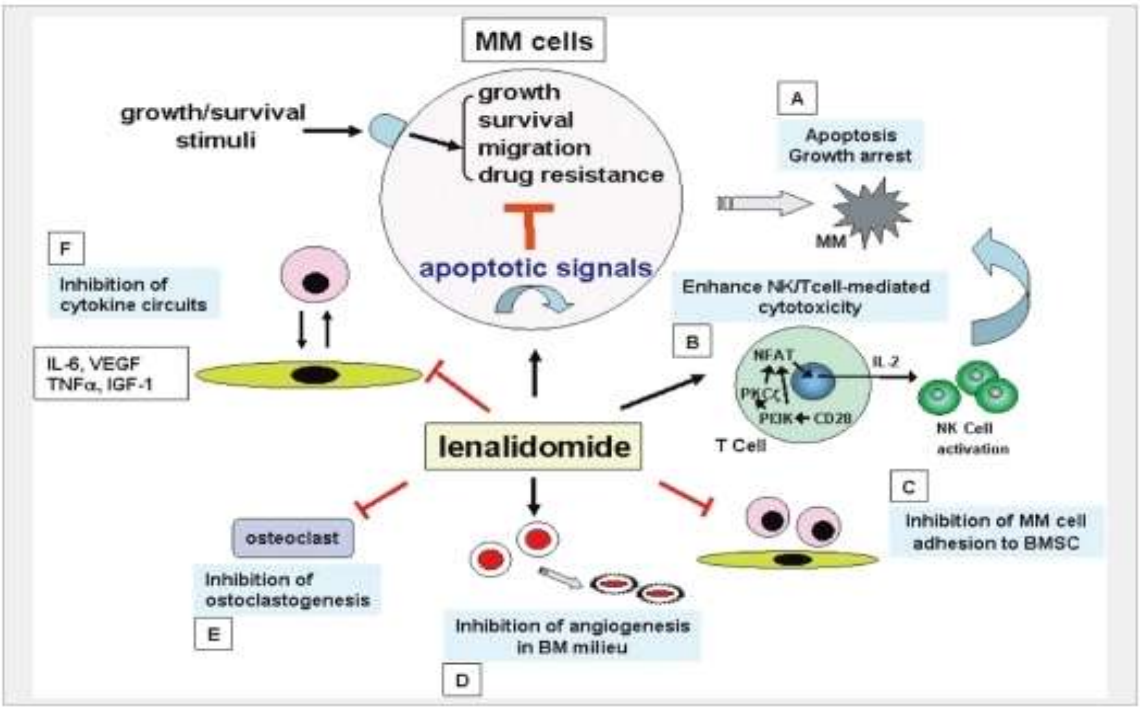


Figure 2.10: Mechanism of action of LND

2.8.3 Challenges and limitations of LND

LND is off-white to pale-yellow powder commercialized under the trade name Revlimid. However, Revelimid hemihydrate (commercial form) due to its inadequate solubility in water has poor oral bioavailability i.e. 33%. Additionally, it has very short half-life i.e. 3 h. Several clinical investigation provide an insight for the utilization of LND in brain-tumor treatment, however clinical trial in phase II is still going on (87,88). Thus LND with other drug makes it a possible candidate for brain tumor therapy. LND possess a limitation that it is not capable of crossing blood brain barrier (BBB) and enter cerebrospinal fluid (CSF) at concentration which

are therapeutically significant. LND additionally goes with atypical biodistribution and extremely short residence time in body. LND therefore need to be administered in such a manner as to improve its brain absorption and the residence at the tumor site.

2.8.4 Nanocarriers for delivery of LND

Nanocarrier based delivery systems reported for LND is summarized in table 2.10.

Table 2.10: Currently developed nanocarriers for delivery of LND

Architecture	Targeting moiety	Particle size (nm)	In-vitro/In-vivo model	Applications	Ref.
LND-Chitosan NPs	-	220-295	MCF-7; U266 cells	LND was 99.35% was encapsulated in chitosan NPs. IC50 value of LND-chitosan NPs against MCF-7 cells was 48.95 µg/ml and against U266 cells it was 24.56 µg/ml	(89)
LND-PLGA NPs	-	179	U266 cells; male wistar rats	The results explained that entrapment efficiency and drug loading was found to be 78% and 32% respectively in PLGA NPsIn-vivo study demonstrated that the bioavailability of LND was enhanced 3.67 folds from PLGA NPs as compared to free LND.	(90)
Fenretinide /LND-nano micelles	-	137.7 - 256.5	SH-SY-5Y NB cells transfected with NTRK2 (clone BR6); female athymic nude mice	The results showed that Fenretinide/LND-nanomicelles demonstrated effective decrease in tumor growth in NB xenograft model.The results displayed that at the end of treatment the tumor mass was found to be almost necrotic with deceased Ki-67 proliferation index.	(91)
pH responsive alloy drug conjugate (SPANs)	HA and Lf		U87 MG cells, in vivo brain uptake study	The in vivo results confirmed enhanced uptake of SPANs in brain after intranasal administration.	(92)

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