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REVIEW ARTICLE

Nanostructured Therapeutic Systems of PUFAs for the Treatment of Glioblastoma Multiforme

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Abstract: Glioblastoma multiforme (GBM) is a typical category of the most common and aggressive brain tumors, with a high incidence in older adults, particularly in males. Although the etiology of GBM has not been fully elucidated, yet it is characterized by highly proliferative activity in the glial cells. Its complete resection is impossible, and radiotherapy is not always efficient for complete relief. Thus, GBM remains a therapeutic challenge in neuro-oncology as there is no treatment that provides significant improvement in the survival rate of patients. In this regard, the identification of newer drug therapy for the treatment of GBM is gaining popularity. However, identifying new targets and developing new leads for screening suitable drug candidates require the investment of resources like time, money, and efforts. It has been observed in many research studies that the use of polyunsaturated fatty acids (PUFAs) as therapeutic moieties for cancer treatment has yielded significant interest owing to their cost-effective availability, limited side effects, and insensitivity towards drug resistance. Nevertheless, the implications of nanostructured therapeutic systems in delivering the PUFAs can provide significant improvement in their biopharmaceutical performance and antitumor activity over the existing alkylating agents used as chemotherapeutic drugs in GBM. Currently, various studies have shown that PUFAs, especially γ-linolenic acid (GLA), have selective tumoricidal action and the ability to reduce antioxidant contents of the glioma tumor cells. In this regard, the present review endeavors to provide an insight into the applications of nanomedicinal drug carriers used for delivering the PUFAs for the effective treatment of GBM and associated diseases.

Keywords: Glioblastoma multiforme, nanostructured therapeutic systems, antitumor activity, PUFAs, neuro-oncology, brain.

1. INTRODUCTION

In 2016, World Health Organization (WHO) upgraded the classification of tumors of the central nervous system in both ways, conceptual and practical [1]. In addition to histology, to explain many tumor units, WHO uses molecular parameters to decide how CNS tumor detection systems should be designed in the molecular era [1]. According to the histological analysis, gliomas can be categorised into low-grade gliomas and high-grade gliomas. Low-grade gliomas (LGG) include grade 1 tumors as slowly proliferative gliomas and grade 2 infiltrative LGG. Third and fourth-grade anaplastic infiltrative gliomas are included under high-grade gliomas (HGGs), referred to as glioblastoma multiforme (GBM) [2]. In predicting prognosis and helping make the best decisions for the treatment, molecular classification plays an important role [2]. In terms of their molecular temperament, GBM is very much heterogeneous and has high genomic instability and intra-tumor variability [3]. Such types of cancers require a "magic bullet" type drug delivery system for reaching the tumor cells.

Various conventional cancer treatment approaches, like chemotherapy and radiotherapy, damage the cancer cells due to their cytotoxic effects [4, 5]. However, there are some limitations associated with these treatments, like using them as modalities of signals in the solid tumor due to the high heterogeneity and cell signaling deregulation [5, 6]. Due to the blood-brain barrier, tumor heterogeneity, and overly aggressive infiltration into the surrounding tissues, GBM is particularly difficult to treat [7]. A combination of various treatment methodologies can be a practical approach for GBM treatment in which different therapies or therapeutic agents with different mechanisms are combined to improve the cytotoxic effects on the cancer cells [8].

Various epidemiological studies have studied the role of natural dietary compounds in cancer metastasis [9]. There is a wide range of natural compounds with antioxidant properties and chemopreventive potential, such as soy isoflavones, curcumin, epigallocatechin, resveratrol, and retinoids [10]. Moreover, various studies have reported that polyunsaturated fatty acids (PUFAs), which are natural bioactive, could kill tumor cells selectively without destroying the normal cells [11, 12-14]. Many PUFAs, like omega-3 fatty acids such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and omega-6 fatty acid (gamma-linolenic acid, GLA), have shown promising efficacy against the glioma tumor cells. GLA exerts a more selective tumoricidal action than other PUFAs (arachidonic acid and eicosapentaenoic acid) [14]. Some *in-vivo* studies have also verified that GLA, eicosapentaenoic acid, and docosahexaenoic acid might have tumoricidal action [15]. In a limited open clinical trial, human gliomas were treated with GLA by intratumoral administration [16, 17]. Various studies have shown that GLA and other PUFAs enhance prostanoid production in cells [18]. To investigate the mechanism(s) by which GLA exerts a cytotoxic effect on astrocytoma cells, we have studied the role of lipid peroxidation or free radical generation, and also of prostanoids and leukotrienes, in the cytotoxic action of GLA and enhancement of the response to radiation. By the action of delta 6-desaturase, linoleic acid (LA, 18:2n6) converts into GLA [19].

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The main objective of this review paper is to describe the role of these natural bioactive in increasing the efficacy of chemotherapeutic drugs in GBM treatment, as reported in many studies.

1.1. GBM Treatment: Current Scenario

GBM treatment is very challenging, as it may cause a differential cellular reaction to specific therapies (tumor heterogeneity) [3]. There are various treatments of GBM, like surgery, chemotherapy, radiation therapy, and combinations of different treatments [20]. The advantages and disadvantages of multiple modalities used for the treatment of GBM are given in Table **1**. GBM cure initiates with surgical resection in those cases where it is possible due to the size and position of the tumor [21]. Surgery plays a dynamic role in the treatment because it reduces tumor mass, thereby decreasing intracranial pressure and providing a histological sample for diagnosis. For controlling symptomatology, surgery is the fastest and the most effective method. With the help of surgery, the efficiency of chemoradiotherapy increases [22]. However, there are cases where complete tumor resection cannot occur because of high proliferative activity and infiltration into the surrounding tissues [23]. In these situations, radiotherapy or chemotherapy is the other solution [24]. The selection of drugs for the treatment of GBM is difficult because of various limitations associated with them, like the blood-brain barrier (BBB), which is an anatomical barrier that protects the brain [25]. This barrier contains essential components, like the ABC transporter family of proteins, which remove foreign substances from cells, including alkylating agents [26]. The size of the tumor is another limitation for better treatment [27]. The size of the tumor must be smaller (around 4 ml) [27]. Furthermore, GBM often shows drug resistance in terms of unregulated signaling pathways, DNA repair pathways, the persistence of cancer stem cell (CSC) subpopulations, and self-defense mechanisms [28]. Currently, the FDA-approved chemotherapeutic agent, temozolomide (TMZ), is preferred for GBM treatment because it is a DNA alkylating agent that prevents DNA replication and destroys cancer cells [21]. Nowadays, intensive research has been carried out for introducing advanced therapies to fight GBM, such as immunotherapy and nanotechnology with natural bio-actives [29]. They give very promising new approaches for GBM treatment to improve the patient's quality of life and survival [30].

1.2. GBM: Molecular Pathology to Targeted Treatment

Targeted molecular therapies can be developed concerning intracellular growth signaling and intercellular signaling, which are usually abnormal in most of the tumors. The first group includes growth factor pathways related to proliferation and apoptosis, constitutively activated in GBM, such as EGFR, PDGFRA, and HGFR/c-MET [31]. For example, in malignant gliomas, PI3K/Akt/mTOR signaling is frequently activated, and due to this, several mTOR inhibitors, like temsirolimus (CCI-779), and everolimus (RAD001), are under evaluation. The second group includes several angiogenic factors, which are required for GBM growth [32]. Of all the VEGF inhibitors, the only one approved by the FDA is Bevacizumab, although other inhibitors, such as Cediranib (AZD2171) and CT-302, have also been evaluated [33]. Despite efforts to develop treatment, the results obtained are not as favorable as expected, with 10-15% response rates or less and no prolongation of survival [34].

1.3. Nanomedicinal Approaches for GBM

As the existing treatment for GBM has several limitations, it is essential to find new approaches for drug prognosis and enhance treatment efficacy [35]. Nowadays, the new strategies include nanomedicines, which comprise nano-sized materials with physical-chemical properties that help in developing new treatments or enhance the efficacy of existing treatments [36].

1.4. Nanoformulations and their Properties

Nanoformulation can transport antitumor agents such as drugs, nucleic acids, or proteins to the tumor cells [37]. In comparison to other drugs, this system also improves the solubility of drugs. There are some other advantages, like the large number of drug nanovesicles that can transport and, in the target tissue, allow controlled and sustained release of the drugs [38]. Nanovesicles enhance the bioavailability by extending their half-life in the bloodstream and protecting the drugs from degradation, metabolism, and renal clearance. Due to passive targeting produced by the enhanced permeability and retention effect, it helps the accumulation of macromolecules in the tumor tissues [39]. However, in the normal tissues, they can reduce the amount of drugs used and the side effects [39]. The therapeutic properties of nanoformulations are typically defined by their physicochemical properties (size, shape, and surface charge) [40]. The size for NPs to be used in nanomedicine usually ranges between 10 to 200 nm [40]. After intravenous administration, nanoformulations, which are very small in size (<5.5 nm), may be eliminated by renal excretion [41]. However, they may be stuck in the spleen sinusoids and liver due to their large size (greater than 200 nm) [40]. The relationship between size and cellular uptake is inversely proportional in tumor cells, which means the smaller the NP, the

Table 1. Different treatment modalities and their advantages and disadvantages.

S. No.	Treatments	Advantages	Disadvantages	References
1.	Surgery	The neurosurgeon will remove the glioblastoma. The goal is to remove as much of the tumor as possible.	As glioblastoma grows into the normal brain tissue, a complete removal is not possible. For this reason, most people receive additional treatments after surgery to target the remaining cells.	[22]
2.	Radiation therapy	Radiation therapy uses high-energy beams, such as X-rays or protons, to kill cancer cells	Radiotherapy can cause side effects, including tiredness, sickness, and loose stool (diarrhoea).	[24]
3.	Chemotherapy	Chemotherapy uses drugs to kill tumor cells. Chemotherapy drugs can be taken in the form of a pill (orally) or injected into a vein (intravenously).	Side effects of chemotherapy depend on the type and dose of drugs you receive. Common side effects include nausea, vomiting, headache, hair loss, fever, and weakness. Some side effects may be managed with medication.	[24]
4.	Targeted drug therapy	Targeted drug treatments focus on specific abnormalities present in the cancer cells. By blocking these abnormalities, targeted drug treatments can cause cancer cells to die.	Vascular and neurologic toxicity.	[24]

greater the internalization [42]. Furthermore, size plays an essential role in greater extravasation in the direction of tumor tissues and the shape of the nanoformulations that may also affect cellular uptake, transport, half-life biodistribution, and degradation [40, 43]. The best form of nanoformulation is spherical; in other words, most synthesized and analysed nanoformulations are spherical. However, oblate particles adhere better to a biological substrate, transport more drugs, and show better adhesion properties in blood vessels. Nonspherical silver nanoformulations present worse biocompatibility, whereas it was found that gold triangle-nanoformulations improved internalization in the mouse macrophage cell line RAW264 [44]. Furthermore, star, rod, and triangle-shaped NPs are internalized in the cells in different proportions of endocytic pathways [45]. Nano vehicles have a surface charge which determines cellular internalization, biodistribution, and cellular interaction. These NPs are positively charged and internalized better than negative or neutral charged particles [46]. The biological properties of NPs can be improved with the help of altering their characteristics and adding definite molecules, which is known as functionalization, improving their cellular internalization, biodistribution, and ability to cross the biological barriers [47].

The diagrammatic depiction of various fundamental properties of nanomaterials is provided in Fig. (**1**).

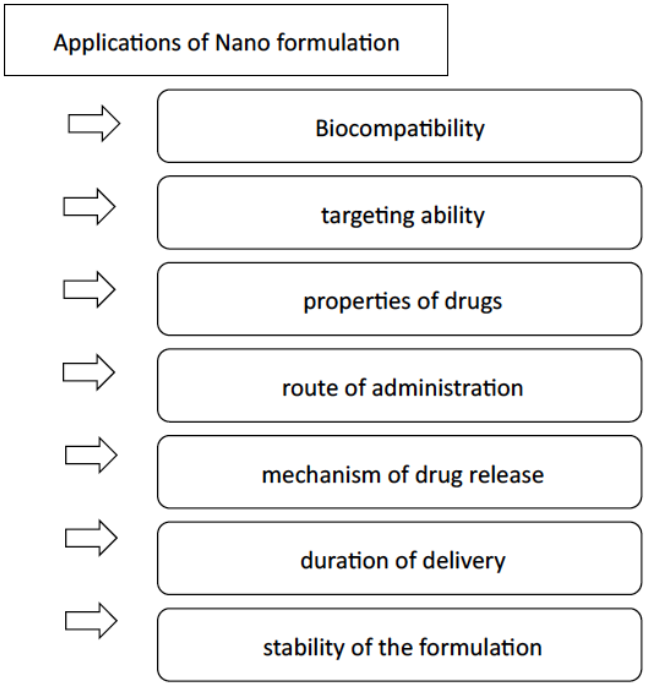

Fig. (1). Overview of various fundamental properties of nanomaterials of brain drug delivery.

1.5. GBM: Active Targeting with NPs

It is challenging for nanomedicine to release sufficient quantities of drugs into the target site, and in GBM conditions, it is very complicated. NPs have to cross the BBB in such types of tumors. Due to the compact brain matrix, the enhanced permeability and retention inhibit the diffusion, resulting in high interstitial fluid pressure. For this reason, passive targeting is insufficient [48]. In the case of GBM, it is essential to find out the target molecules that can cross the BBB and the blood-brain tumor barrier (BBTB) [49]. Therefore, the entrance of antitumor drugs to the brain for GBM treatment is very restricted; however, tiny molecules (<400 Da and <8 hydrogen bonds) cross the BBB *via* lipid-mediated free diffusion [50]. Within the brain tumor tissues, the structure and function of the BBB are altered and transformed into the blood-brain tumor barrier (BBTB) through angiogenesis. The new vasculature of the BBTB is heterogeneous and has differences in its permeability [51]. Notwithstanding, this heterogeneity does not enhance the efficiency of antitumor drugs [52]. With the help of existing therapeutic approaches based on drugs, enzymes, DNA or RNA molecules, antioxidant or inorganic molecules encapsulated within polymeric or lipid nanoformulations can be used to cross the BBB or BBTB in GBM [52]. Without the addition of specific ligands or coatings, lipid-based nanoformulations are very efficient and capable of crossing the BBB [53]. Still, their applicability is restricted due to their low loading capability and gelation tendency. On the other side, polymeric NPs show great resourcefulness and the possibility of binding ligands to functionalize nanoformulations [54]. Among the overexpressed targets in GBM, and commonly used target molecules in nanomedicine, are the LRP1, TfR, GLUT, αvβ3 integrin, GFAP Cx43, EGFR, EGFRvIII, IL-13Rα2, and Fn14 receptors [55].

1.6. Nanoformulations, their Structure and Activity for GBM Treatment

The instances of some of the extensively employed nanoformulations for GBM treatment have been described in the below sections and pictorially illustrated in Fig. (**2**). Also, Table **2** enlists various instances of nanocarriers used for the clinical treatment of GBM.

1.6.1. Liposomes

Liposomes are nanocarriers, which are composed of one or more aqueous cores surrounded by a lipid bilayer into which therapeutic agents are transported in the form of hydrophilic agents [56]. The external membrane, which is made up of lipid, hydrophobic molecules, may also be incorporated. Also, negatively charged DNA or RNA molecules can be incorporated into cationic liposomes. They possess high biocompatibility and low toxicity to cross the BBB in the treatment of cancer [57].

1.6.2. Solid Lipid Nanoparticles (SLNs)

SLNs consist of nanoparticles of solid lipids prepared from a lipid matrix at room temperature and stabilized by surfactants. Various lipids are used for designing the SLNs, like Compritol® 888 ATO, Precirol® ATO5, cetyl alcohol, tripalmitin, trimyristin/Dynasan® 114, tristearin/Dynasan® 118, stearic acid, glyceryl monostearate, and cetyl palmitate. SLNs have many advantages, like the physical stability that protects the encapsulated drugs, enhanced targeting with the help of surface modification, controlled release characteristics, and excellent biocompatibility, helping them escape more easily from the reticuloendothelial system [58]. It has been observed that SLNs can cross the BBB. According to Huang *et al*. [59], TMZ- loaded SLNs increased the brain targeting efficiency and reduced the toxicity in mice compared to free TMZ. Another study also showed that SLNs composed of cetyl palmitate, coated with surfactants (polysorbate 60 and 80), and loaded with camptothecin (CPT), showed excellent ability to cross the BBB. These SLNs enhanced the cytotoxicity of the drug in U373 cancer cell lines. Compared to free drugs, they enhanced the drug's bioavailability (24 *vs*. 8 hrs), which was analyzed by HPLC. There are some limitations of SLNs, such as low drug loading capacity, a polymorphic transformation of drugs to the crystalline structure, aggregation, and unpredictable gelation tendency [58]. The angiopep-2 peptide was grafted on some surface-modified SLNs, recognized *via* LRP1, showed greater ability to cross the BBB, and internalized into the GBM tumor cells [60]. In a study, these SLNs loaded with docetaxel showed an increased docetaxel efficacy due to better accumulation in the glioma tumor-bearing mice (four-fold increase) compared to the free docetaxel [61].

1.6.3. Nanostructured Lipid Carriers

Nanostructured lipid carriers (NLCs) have developed as a new generation of SLNs to improve the limitations of existing SLN.

Table 2. Various nanocarriers used for delivering drugs for the treatment of GBM.

S. No.	Nanotechnology	Drug Delivery	References
1.	Liposomes	Doxorubicin-loaded liposomes functionalized with the ligands pHA and cyclic peptide c(RGDyK) facilitate the crossing through the blood-brain barrier and the blood-tumor brain barrier. These liposomes designed for the treatment of glioblastoma multiforme are composed of hydrogenated soy phosphatidylcholine, cholesterol, and mPEG2000-DSPE and are synthesized by thin-film hydration and extrusion method.	[57]
2.	Solid lipid nanoparticles	Camptothecin-loaded solid lipid nanoparticles have demonstrated the ability to cross the blood-brain barrier *in vitro* and *in vivo*. These nanoparticles, composed of cetyl palmitate and polysorbate 60 surfactants, are synthesized by high shear homogenization and ultrasonication techniques.	[58]
3.	Polymeric NPs	PTX with PEG form NPs, which are associated with a ligand for neurokinin-1, the substance P peptide. *In vitro*, these are overexpressed in tumors such as GBM, resulting in a decreased IC_{50} for PTX and improved uptake in BCECs and U87 cells. *In vivo*, at the tumor site, these NPs display greater accumulation, associated with a higher antitumor effect and low systemic toxicity.	[67]
4.	Polymeric micelles	cRGD peptide-conjugated poly(β-ethylene glycol)-copoly(lactic acid) PM was functionalized with a peptide antagonist for p53 negative regulators MDM2 and MDMX (sPMI), improving the ability of p53 activators to cross the BBB and displaying a better tumor growth inhibitory effect in combination with TMZ in intracranial U87MG nude models.	[71]
5.	Polymersomes	Figueiredo *et al*., in 2016,synthesized a poly(dimethylsiloxane)-poly(2-methyloxazoline; PDMS-PMOXA-based Ps) functionalized with Angiopep2 and loaded with DOX. This PDMS-b-PMOXA-Angio-pep2 Ps showed an enhanced cellular uptake in U87MG cells compared to PDMS-b-PMOXA due to the overexpression of the LDLP-1 that occurs in glioma cells.	[76]
6.	Dendrimers	A nonviral vector system was developed to successfully improve the delivery of two siRNAs (VEGF and B-cell lymphoma/leukemia-2 [Bcl-2] siRNAs) to GBM cells.	[81]
7.	Nanogels	Shatsberg *et al*., in 2016, designed a polyglycerol-scaffold based nanogel to direct miR-34a in the treatment of GBM, leading to high expression levels of miR-34a in U-87 MG cells, and in this way achieving remarkable downregulation of miR-34a target genes (involved in apoptosis and cell cycle arrest).	[84]
8.	Silica NPs	pH-responsive CS-coated MSNs were loaded with curcumin to treat U87MG glioblastoma cells, showing a significant reduction in the drug IC_{50} (up to 2.9-times).	[90]
9.	Carbon nanotubes	According to the study, functionalized MWCNTs with angiopep-2showed an increase in the passage of the drugs targeting through the BBB. These formulations were used to control drug kinetics release in GBM treatment with the encapsulation of PTX and dasatinib.	[94]
10.	Nano-graphene	According to the study, with the help of a pegylated nanoformulation with arginine-glycine-aspartic acid peptide Balb/c, mice bearing subcutaneous U87MG tumors were treated with reduced graphene oxide nanomesh (rGONM).	[100]
11.	Nanorods and nanowires	For early tumor detection in GBM, some studies have used gold nanorods. To detect angiogenic endothelium and malignant glioma cell surfaces, GNRs connected to cyclic Arg-Gly-Asp (cRGD) peptides were used. The efficacy of GNRs from human GBM was demonstrated, which was associated with PEG methyl ether thiol and peptide fluorescently for Nestin to mark CSCs (spheroids).	[110, 111]
12.	Quantum dots	With the help of intravenous QDs, C6 glioma cells were intracranially implanted in rats. This study showed that the use of QDs in the surgical resection of brain tumors, like GBM, serves as an optical aid for demarcating the tumor area.	[116]
13.	Magnetic NPs	Magnetic H_3O_3P-Fe_3O_4 NPs were synthesized with gH625 peptide (derived from the glycoprotein H of the HSV-1) or PEG-folic acid molecules to favor the passage of MNPs through the BBB, demonstrating that both systems can be taken up by immortalized human brain microvascular endothelial cells and brain tumor cell lines (*i.e*., glioblastoma A-172).	[120]

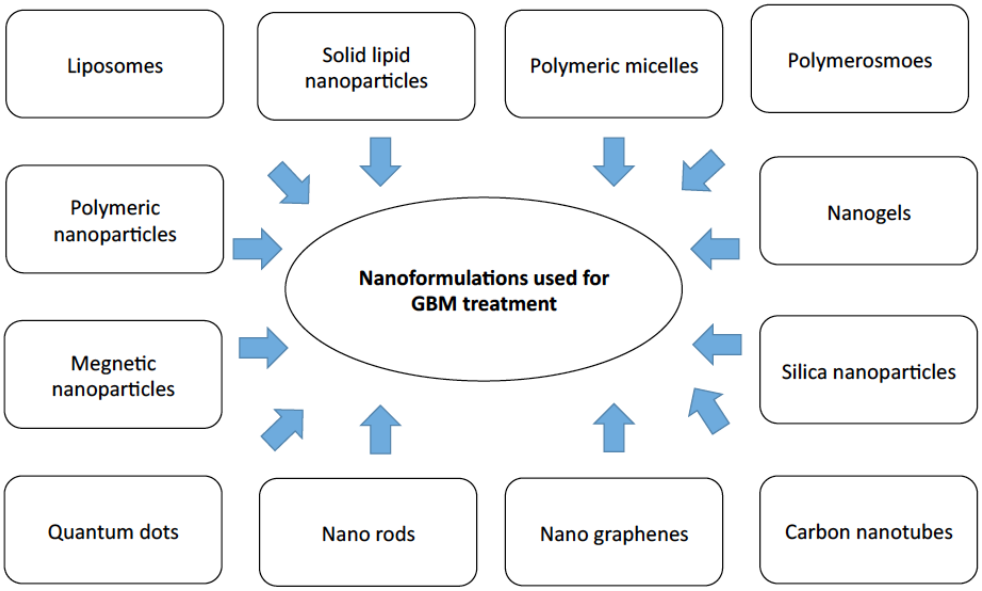

Fig. (2). Instances of various nanocarriers studied in the literature for GBM treatment.

At room temperature, NLCs are a mixture of both lipids (liquid and solid) with a crystalline structure, which enhances the loading capacity of drugs and reduces drug expulsion during storage [58]. Polymeric NPs (PNPs), SLNs, and NLCs with the TMZ loaded nanoformulations were compared in U87 malignant glioma cells [62]. It has been observed that the IC_{50} value of TMZ-loaded SLNs and TMZ-loaded PNPs was four and seven times greater than TMZ-loaded NLCs, respectively. Moreover, in nude mouse models, TMZ-loaded NLCs induced a more significant volume reduction (85%) in U87 tumors than SLNs and PNPs(59% and 45%, respectively). These studies have recommended that this new formulation could be a potential strategy for glioblastoma treatment [61].

1.6.4. Polymeric NPs

Polymeric NPs incorporate therapeutic agents into their polymeric matrix or on their surface where conjugate is present, and with the help of biocompatible polymers, polymeric NPs are synthesized. These NPs can entrap drug molecules and help in resolving drug delivery challenges, such as drug insolubility in water and short *in vivo* lifetimes [63, 64]. The FDA has approved the PLGA, and it is the most commonly used polymer because of its biocompatibility. However, nowadays, for the synthesis of NPs, PEG is being used in combination with PLGA, leading to the development of a PLGA-b-PEG copolymer. According to a previous study [64], a multifunctional nanoformulation of silver (PLGA-b-PEG) NPs conjugated with a chlorotoxin (binds to MMP-2) and the drug alisertib achieved a synergistic effect between both agents, and a tumor reduction was observed in *in vivo* models [65]. For the treatment of GBM, the most significant natural polymers used to create NPs are based on polysaccharides. For example, chitosan, hyaluronic acid, corn starch, and alginate, and proteins include albumin, gelatin, collagen, transferrin, lactoferrin, and silk fibroin [65, 66, 67]. They develop albumin-based NPs *via* covalently bonding human serum albumin (BSA) molecules, improving stability and drug absorption. NPs involving PTX with PEG are associated with a ligand for neurokinin-1, the substance-P peptide. Under *in vitro studies*, their overexpression in tumors such as GBM resulted in a decreased IC_{50} for PTX and improved uptake in BCECs and U87 cells. Under *in vivo* studies, these NPs display more significant accumulation at the tumor site, exerting a higher antitumor effect and low systemic toxicity [68]. Chitosan-based NPs loaded with PTX and coated with 1,3 β-Glucan were synthesized to decrease the hemolysis caused during the intravenous administration of PTX and reduce systemic toxicity while increasing cytotoxicity in LN18 and C6 glioma cells [68].

1.6.5. Polymeric Micelles

Polymeric micelles (PMs) comprise two or three segments of amphiphilic block copolymers (hydrophilic-hydrophobic and hydrophilic-hydrophobic-hydrophilic, respectively). PMs are minute particles that load and deliver therapeutic agents (drugs, proteins, or nucleic acids) to malignant cells [64]. They have design flexibility, lower CMC (critical micelle concentration), better stability, a hydrophobic core, and an aqueous environment (with the therapeutic agent). However, these nanoformulations can only encapsulate hydrophobic agents, which is their major limitation [69]. There are some examples in which various PM formulations incorporate the cRGD peptide on their surface, enhancing the transport of PM and therapeutic agents through the BBB. One such example is cRGD-linked PMs loaded with (1,2-diaminocyclo-hexane)platinum(II) (DACHPt), the parent complex of oxaliplatin [70]. Other systems worth mentioning are cRGD peptide-conjugated poly(β-ethylene glycol)-copoly(lactic acid) PM functionalized with a peptide antagonist for p53 negative regulators MDM2 and MDMX (sPMI), which improves the ability of p53 activators to cross the BBB and displays a better tumor growth inhibitory effect in combination with TMZ in intracranial U87MG nude mice models [71]. Besides, cRGD-PMs conjugating epirubicin (cRGD-Epi/m), an anthracycline with poor penetration ability, and P-glycoprotein substrate effectively suppress growth in an orthotopic GBM model *in vivo* [72].

Other studies have indicated that functionalized PMs with choline derivatives or GLUT1 variable single-chain fragment (scFv) can enhance both cellular uptake and the accumulation of PMs at the tumor site, permitting drugs such as DOX to pass the BBB, in addition to slowing down its elimination [73, 74]. In the latter, it is important to highlight the synergistic effect of GLUT1-scFv-PEGPE-based PMs co-loaded with DOX and curcumin against the U87MG cells, which derived from glioblastoma [74]. Currently, some studies have been carried out to synthesize PMs. According to a study [75], it was found that in intracranial GBM models *in vivo,* deoxycholic acid-conjugated polyethyleneimine PMs loaded with curcumin and an antisense-oligonucleotide against miR-21 (miR21ASO) shown promising therapeutic effects. Curcumin was able to decrease tumor volume in addition to

restoring the expression of the PTEN and PDCD4 genes silenced by microRNA-21.

1.6.6. Polymersomes

Polymersomes (Ps) are also self-assembled polymeric vesicles composed of synthetic amphiphilic or ionic block copolymers. In terms of structure, the polymersome is similar to a liposome. In the Ps, a large hydrophilic cavity is present and can encapsulate both types of molecules and hydrophobic layers present. However, these synthetic bilayer polymers are more stable to shear stresses than lipid membranes. Moreover, their chemical configuration can provide various properties, like high mechanical stability, elastic behavior, higher membrane viscosity, biodegradability, and biocompatibility [64, 69, 76]. PEG-conjugated (PEGylated) polyion complex vesicles (PICsomes), a novel class of polymersomes, originate the self-assembly of PEG-based block aniomers and homo-catiomers through the electrostatic interaction forces [77]. These PICsomes were conjugated with cRGD peptides on the distal end of PEG strands allowing active neovascular targeting. The results revealed that 40%-cRGD-PICsomes (PICsome with 40% PEG distal end substituted by cRGD moieties) accumulated mainly in the tumor neovasculature *in vivo*, making them a promising tool in tumor imaging for an accurate diagnosis. The targeted Ps using a methacryloylated cholesterol derivative as the hydrophobic block and 2-hydroxyethyl methacrylate as the hydrophilic block were functionalized with a BBB-specific peptide (ligand of LRP-1), allowing the system to cross a BBB model [76]. Figueiredo *et al.* developed a poly(dimethylsiloxane)-poly(2-methyloxazoline) PDMS-PMOXA-based Ps functionalized with Angiopep2 and loaded with DOX. These PDMS-b-PMOXA-Angio-pep2 Ps showed an enhanced cellular uptake in U87MG cells compared to PDMS-b-PMOXA due to the overexpression of the LDLP-1 that occurred in glioma cells. DOX was encapsulated using the co-solvent method, showing a slow release rate from the Ps that could protect the drug from hydrolyzation. Recently, it has been observed that ANG polymersomes (angiopep-2-directed/redox-responsive virus-mimicking PEG-b-poly(trimethylene carbonate-co-dithio-lanetrimethylene carbonate)-b-polyethyleneimine) can selectively guide saporin, a natural cytotoxic protein that only exerts an effect within cells, against GBMs developed in mice [78]. ANG-PS-SAP displayed a remarkable antitumor activity towards U-87 MG human GBM cells *in vitro* and high GBM accumulation *in vivo* [78].

1.6.7. Dendrimers

Dendrimers have a core surrounded by various branches comprised of many functional groups, which are artificial macromolecules. Several dendrons (branching units) arising from the core are arranged in a symmetric manner [64, 79]. For effective drug delivery, the size and shape of dendrimers can be modified. The most widely used dendrimers in biomedicine are PAMAM dendrimers because they are hyperbranched, exist in 3D structure, and have well-defined molecular weight and entrapment properties. They have various functional end groups because of their high solubility and reactivity due to a high amino group density [80].

In recent times, various therapeutic strategies have been conceded out with PAMAM dendrimers with favorable results. A nonviral vector system has been designed to improve the delivery of two siRNAs (VEGF and B-cell lymphoma/leukemia-2 (Bcl-2) siRNAs) to GBM cells successfully [81]. Additionally, a system of PAMAM dendrimers with conjugated three peptides (cytotoxic peptide (KLAK), cell-penetrating peptide (TAT), and MMP2-sensitive peptide] has also been developed, which are observed to improve the penetration into tumor cells and enhance the targeting specificity [82].

1.6.8. Nanogels

The new class of nanosystems is nanogels. Nanogels have macromolecular structures resulting from 3D cross-linking and are characterized by their softness. For the encapsulation, this structure of nanogels allows several bioactive components, like drugs, proteins, peptides, and DNA/RNA, and in cell lines and organs stimulates the drug release. A system of nanogels loaded with cisplatin and conjugated using PEG and polymethacrylic acid block copolymer (PEG-b-PMAA) with monoclonal antibodies to Cx43 (MAbCx43) through flexible PEG bridges was designed [83]. Increased accumulation in C6 glioma cells was observed compared to nanogels withoutMABCx43. Furthermore, an improved survival and tumor cell growth inhibition with an induced C6 intracranial glioma in rats were observed. Shatsberg *et al*. designed a polyglycerol-scaffold-based nanogel to direct miR-34a in the treatment of GBM, leading to high expression levels of miR-34a in U-87 MG cells and remarkable downregulation of miR-34a target genes (involved in apoptosis and cell cycle arrest). A dual-loading drug delivery system of folate-CS (FA-CS) nanogels with anionic gold NPs (AuCOOH@FACSnanogel system) for the intracellular co-delivery of TMZ and miR-218 mimics in glioma cells was developed [84]. The results have shown a synergistic effect against U87MG GBM cells.

1.6.9. Silica NPs

In the field of nanomaterials studied, silica NPs exhibit unique properties. Silica NPs have good biocompatibility, a large surface area, and flexible pore size. They are used to increase the biocompatibility of other nanoformulations in the nervous tissue, such as iron oxide NPs [85]. For the transport and release of drugs, Mesoporous silica NPs (MSNs) are also used in GBM treatment [86]. According to the recent experiment [87], MSNs have shown the maximum permeability through BBB with a size of about 40 nm. Moreover, the acid pH may be efficient stimuli to enhance the drug release, like in pH-responsive MSNs loaded with CPT and conjugated with DOX and the folic acid-coated MSNs loaded with valproic acid [88, 89]. Recently, pH-responsive CS-coated MSNs have been loaded with curcumin to treat U87MG glioblastoma cells, showing a significant reduction in the drug IC_{50} (up to 2.9-times) [90].

Silica NPs have played an essential role in altering the signaling pathways, such as VEGF receptor (VEGFR) associated with angiogenesis and tumor metastasis. An anti-VEGFR ligand (VEGF121) loaded to pegylated MSNs was designed to treat U87MG glioblastoma-bearing mice [91]. Moreover, silica NPs were used to improve toxic radiotherapy effects, such as folic acid-coated MSNs loaded with valproic acid, enhancing the glioma cell lines C6 and U87MG radiosensitivity. Due to this, an increase in cell death was observed, which allowed a significant reduction in the radiation dose and, therefore, lowering the toxicity in the nearby tissues [89]. Finally, when neural stem cells were used to transport NPs against glioma tissue, silica NPs (MSN loaded with DOX) were found to be useful in retarding the toxicity of the drug in these stem cells [92]. Most DOXs (96%) were observed in the orthotropic human glioma xenograft after 4 hours of inoculation, increasing the antitumor effect and mice's survival [92]. According to some studies, silica NPs signify a specific cell type toxicity because some studies have observed that silica gel is toxic for GBM LBC3, LN-18, and LN229 cell lines but not for human skin fibroblasts, whose mechanisms have not yet been clarified. Therefore, more research is needed in the area of the tumor and nontumor CNS cells [61].

1.6.10. Carbon Nanotubes

Carbon nanotubes (CNTs) are cylindrical nano-sized structures made of carbon atoms systematized into aromatic rings. They can be classified into two parts based on their layers, one is single-walled CNTs (SWCNTs), or another one is multi-walled CNTs (MWCNTs) [93]. Due to their tunable physical and chemical properties, CNTs have an excellent capacity for carrying drugs to treat GBM tumors as they have the ability to cross the BBB [94]. Antitumor drugs may be transported in the external sidewalls or in

the inner cavities, where they are more protected and leave the outer part of the nanotube free to be functionalized [93]. According to a study, functionalized MWCNTs with angiopep-2 showed an increase in the passage of drugs targeting through the BBB [94]. These formulations are used to control drug kinetics release in GBM treatment with PTX and dasatinib [95]. CNT formulations may show toxicity in the brain. Due to impurities, size, and surface characteristics, the cell model was used [96]. In a research study, four different CNT formulations were found to be toxic in both RG2 glioma cells and normal astrocytes [97]. . Another study showed that CNTs were able to modify the neurotoxicity of intraperitoneally administered SWCNTs and MWCNTs in mice. They altered the expression of the BDNF and induced behavioral toxicity in mice. However, the toxicity produced by MWCNTs was higher than that of SWCNTs [98].

1.6.11. Nano-graphenes

Graphenes comprise carbon atoms in the layer structure and are considered a 2D material, having regular hexagonal patterns [99]. Graphenes have a large surface area and many functional groups for transporting molecules, making them an applicant for nanomedicine, including GBM treatment. Graphenes and nanoformulation have given promising results. According to the study, with the help of a PEGylated nanoformulation with arginine-glycine-aspartic acid peptide, Balb/c mice bearing subcutaneous U87MG tumors were treated with reduced graphene oxide nanomesh (rGONM) [100]. Instead, in the GBM cell line of U87, poly(amidoamine)-dendrimer and gadolinium-functionalized nanographene oxide associated with epirubicin and nucleic acids (Let-7g miRNA, an inhibitor of Ras oncogene family expression) showed significant antitumor effect [101]. Furthermore, the oxidized graphene nanoribbons associated with Lucanthone exhibited specific toxicity in the GBM cell line U251 compared to the other tumor cell lines (MCF-7) and nontumor glial cell lines (CG-4) [102]. One use of graphene oxide is to attack tumor cell organelles, like mitochondria [103]. Claudin 4-targeted graphene oxide linked to the photodynamic agent chlorin e6 generates ROS when excited, increasing the temperature and causing death in the claudin-4 overexpressing U87 GBM cell line [104]. Chlorotoxin-loaded DOX with graphene oxide sheet increases DOX cytotoxicity in C6 GBM cells [105]. A study on graphene oxide functionalized with PLGA to transport 5-iodo-2-deoxyuridine (IUdR) under NIR (near-infrared laser) and radiation (x-ray) showed significantly higher cytotoxicity in the U87 GBM cell line than the other methods alone [106]. Adding proline or arginine into graphene solution may increase the graphene nanoformulations cytotoxic activity in the U87 GBM cell line [107]. Lastly, the combination of graphene oxide and DOX reduced photothermal activity, thereby increasing the survival (40 days) of U87MG tumor-bearing mice compared to those treated without irradiation (14-20 days) [108].

1.6.12. Nanorods and Nanowires

For tumor treatment, nanorods are a new type of NPs which have a size between 1 and 100 nm and are produced from semiconducting materials or metals. There are various types, but gold nanorods (GNRs) exhibit low toxicity. They are used as contrast agents (photoacoustic and near-infrared imaging of tumors) and are utilized in photothermal therapy [109].

For early tumor detection in GBM, some studies have used gold nanorods. To detect angiogenic endothelium and malignant glioma cell surfaces, GNRs connected to cyclic Arg-Gly-Asp (cRGD) peptides were utilized [110]. Recently, the efficacy of GNRs from human GBM associated with PEG methyl ether thiol and peptide fluorescently for Nestin to mark CSCs (spheroids) has been successfully demonstrated [111]. Polymeric NPs containing GNRs used in nude CD1 mice with glioblastoma tumors showed a significant reduction in the tumor size [112].

On the other hand, within the category of nano neuromodulators, nanowires are structures with diameters of only a few nanometers and extended lengths, which are principally used for diagnostics purposes. Without the use of a probe, nanowires can monitor brain electrical activity. Nanowires may be developed from silicon, germanium, carbon, gold, and copper [113]. Only a few studies demonstrate the role of the nanowire in GBM tumors. For example, zinc oxide nanowires were conjugated by green fluorescent peptides to image U87MG human glioblastoma cells *in vivo*, suggesting an innovative nano platform for cancer imaging and therapy [114]. To elucidate the importance of nanorods and nanowires, further studies are needed in GBM treatment.

1.6.13. Quantum Dots

Quantum dots (QDs), due to their size (2-10 nm), have unique optical and electrical properties. These QDs, which are optical semiconductor nanocrystals, are easily detectable because they only emit light at a characteristic wavelength. They can absorb photons over a wide range of wavelengths [115]. It has been observed that with the help of intravenous QDs, C6 glioma cells were intracranially implanted in rats [116]. This study has shown that using QDs in the surgical resection of brain tumors, like GBM, serves as an optical aid for demarcating the tumor area [116].

To generate quantum dot-containing immunoliposome hybrid NPs, QDs can also be conjugated with molecules, like antibodies (anti-EGFRvIII single domain) or other NPs [117]. NPs help in enhancing the *in vitro* internalization, resulting in therapeutic benefits for GBM treatment, as observed during *in vivo* and *in vitro* studies. For the future application of gene therapy, various studies have shown how QDs can be used as a tool against brain cancer [118].

1.6.14. Magnetic NPs

Magnetic iron NPs (MNPs) have gained much interest in diagnostic and therapeutic (theranostics) applications. These and have versatile surface functionalization and biocompatibility due to their magnetic characteristics. For MNP synthesis, maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4) are the two FDA-approved magnetic materials; the first offers the best intrinsic magnetic response for biomedical applications. Surface functionalization with carboxylic acid (-COOH) or amine (-NH_2) functional groups is required to incorporate targeting ligands to further surface conjugations to prevent surface oxidation and agglomeration and improve biocompatibility. There are various applications of MNPs such as cell labeling, sorting, and manipulation, magnetic guidance for drug delivery, hyperthermia, and magnetic resonance imaging (MRI) [119, 120]. The magnetic field is an important factor for the remote control to cross the BBB. It makes them promising nanostructures for brain cancer therapy. However, the magnetic force produced may not be enough to disrupt key cell components due to their small size. New approaches have been established to improve MNP response to magnetic fields by incorporating Zn, Ni, and Co metal elements into the NPs to improve the magnetization [121]. Magnetic H_3O_3P-Fe_3O_4 NPs with gH625 peptide (derived from the glycoprotein H of the HSV-1) or PEG-folic acid molecules were synthesized to favor the passage of MNPs through the BBB, demonstrating that both systems can be taken up by immortalized human brain microvascular endothelial cells and brain tumor cell lines (*i.e.*, glioblastoma A-172) [120]. Zinc-doped iron oxide NPs functionalized with EGF peptide were developed and directed to the target/tumor site under a low-frequency rotating magnetic field [121]. After rotating magnetic field exposure, NPs form elongated aggregates, severely damaging the cell membranes due to the torque generated. However, they are more frequently associated with chemotherapeutic agents generating multifunctional nanoplatforms. For example, MNP-PEG-coated with dialdehyde O6-benzylguanosine, an MGMT analog inhibitor, achieved a pH-dependent drug release suppressed by MGMT *in vitro* and promoted apoptosis when combined with TMZ [122]. Also, remotely FA-grafted CS-coated MNPs loaded with

DOX significantly decreased the tumor growth in mice with subcutaneous tumors developed using GBM U87 cells under magnetic guidance [122]. Another widely studied field involves the use of MNPs in MRI. Numerous authors have associated these systems with molecules such as caffeic acid or monoclonal antibodies against VEGF (mAbVEGF) to improve their biodistribution and *in vivo* targeting [123]. During cancer therapy, it is desired that target therapeutic agents preferentially kill tumor cells without killing the normal cells. In this context, the anti-cancer and anti-angiogenic actions of gamma-linolenic acid (GLA) and its derivatives look promising [123].

1.7. *In vitro* Studies on Polyunsaturated Fatty Acids (PUFAs) with Tumoricidal Action

All PUFAs cannot be synthesized by mammalian cells; therefore, it is widely distributed in human food, like essential fatty acids, linoleic acid, and alpha-linolenic acid. Both linoleic acid and alpha-linolenic acid are desaturated and elongated in structure. Thus, linoleic acid forms gamma-linoleic acid (18:3 o-6), dihomo gamma-linoleic acid (DGLA, 20:3 o-6), and arachidonic acid (AA, 20:4 o-6), whereas alpha-linolenic acid forms eicosapentaenoic acid (EPA, 20:5 omega-3 fatty acid) and docosahexaenoic acid (DHA, a 22:6 omega-3 fatty acid). The omega-6 EFA metabolism is found to be irregular. The omega-3 PUFAs (made from ALA) concentrations in tumor tissues are not modified (such as EPA and DHA) [124]. Moreover, EPA and DHA can be found in marine foods. According to Das and co-workers, cis-unsaturated fatty acids, such as GLA and arachidonic acid, kill tumor cells without harming the normal cells [125-128]. In mixed culture (both normal and tumor cell) experiments, GLA showed a more selective tumoricidal action than others [14]. Some *in vivo* studies have also demonstrated that GLA, EPA, and DHA are capable of killing the tumor cells [129, 130]. Due to the difference in PUFA metabolizing enzymes expression, *in vivo* GLA metabolism in humans is extremely complex because all cellular compartments do not metabolize it uniformly. Besides apoptosis in glioma tissue infused with GLA for 3-7 days, the preservation of normal brain tissue and vasculature in the adjacent brain with little inflammation as evidence in regressing tumors was noticed in the glioma infusion model. This study indicated that GLA intraparenchymal infusion is effective in stimulating glioma regression [131].

1.8. PUFAs: Mechanism of Tumoricidal Action

PUFAs have shown increased tumor cells due to improved free radical generation and lipid peroxidation [12, 132]. Due to the enhancement of free radical generation and lipid peroxidation, uptake of PUFAs was at least two to three times greater in normal cells than tumor cells [133]. Some PUFAs, like GLA, at appropriate concentrations showed tumoricidal actions during the *in vitro* studies without affecting the normal cells [12-14, 126, 127]. This action of PUFAs is effective in killing the tumor cell and cannot be blocked by inhibitors of cyclooxygenase (COX) and lipoxygenase (LO) [12, 14]. However, the tumoricidal action of PUFAs is blocked by other antioxidants, like vitamin E, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), indicating that free radicals and lipid peroxides are involved in their tumoricidal activity [14, 134]. PUFAs induce tumor cells apoptosis, causing DNA strand breaks and reducing the antioxidant content of tumor cells significantly [135, 136]. It has been observed that GLA and other PUFAs enhance the expression of P53 and suppress the oncogene ras, Bcl-2, an anti-apoptotic gene [137]. GLA was found to be the most effective of all the PUFAs tested. GLA and other PUFAs produced modifications in the lipid composition of tumor cell membranes [138] and mitochondrial ultrastructure and improved responsive oxygen species (especially superoxide anion, O2-.) and lipid peroxides production. GLA-treated tumor cells showed a reduction in the mitochondrial respiratory chain complexes I + III and IV activity and the potential of the mitochondrial membrane. Mitochondria released the cytochrome C and activated the caspases and DNA fragmentation [139], leading to the apoptosis of tumor cells. Tumor cells produced huge volumes of 2 sequences of prostaglandins (PGs), which may promote their growth [140]. EPA and DHA suppressed the production of 2 series of PGs and, therefore, inhibited tumor growth. Inhibition of tumor growth with the help of EPA/DHA was not affected by COX-1 or COX-2 overexpression, showing that omega-3 fatty acids were not dependent on the COX enzyme [141]. These results are similar to GLA, in which it was noted that COX or lipoxygenase inhibitors did not block the tumoricidal actions of GLA [142]. Table **3** summarizes the various mechanisms of action of the PUFAs used for the treatment of GBM.

1.9. Role of GLA in the Free Radicals and Lipid Peroxides

According to the observation of GLA cytotoxic action on HeLa cells, enhanced superoxide and generation of H_2O_2 and higher lipid peroxides development in both HeLa and ZR-75-1 cells were observed [12, 126, 143a-c]. However, various studies suggesting that EPA and DHA also enhanced superoxide and H_2O_2 generation and the formation of lipid peroxides in HeLa cells and human breast cancer (ZR75-1) cells discovered that GLA has the property to protect the normal cell and affect only the cancerous cells (human skin fibroblasts, CCD-41-SK) [12, 126].

Table 3. Summary of the mechanism of actions of PUFAs.

S. No.	Mechanism of Actions	References
1	PUFAs might influence the concentration of hormones, thereby influencing the cell and tissue actions.	[133]
2	PUFAs might influence other factors, like low-density lipoprotein oxidation, oxidative stress, *etc*., that in turn impact cell and tissue behavior.	[138]
3	Polyunsaturated Fatty Acids (PUFAs) and their metabolites are natural ligands for PPAR (peroxisome proliferator-activated receptor) gamma, which regulate inflammatory gene expression and activation of NFKB. Activation of PPAR alpha is also related to COX-2 expression induction. They may also decrease triglyceride synthesis because they are poor substrates for the enzymes responsible for TG synthesis and prevent other fatty acid esterification.	[141]
4	PUFAs also reduce the adhesion expression of the molecules on leukocytes and endothelial cells and reduce intercellular adhesive interactions.	[142]
5	PUFAs are also reported to directly activate syntaxin-3, a plasma protein membrane that controls the transport of vesicle and neurites growth.	[142]

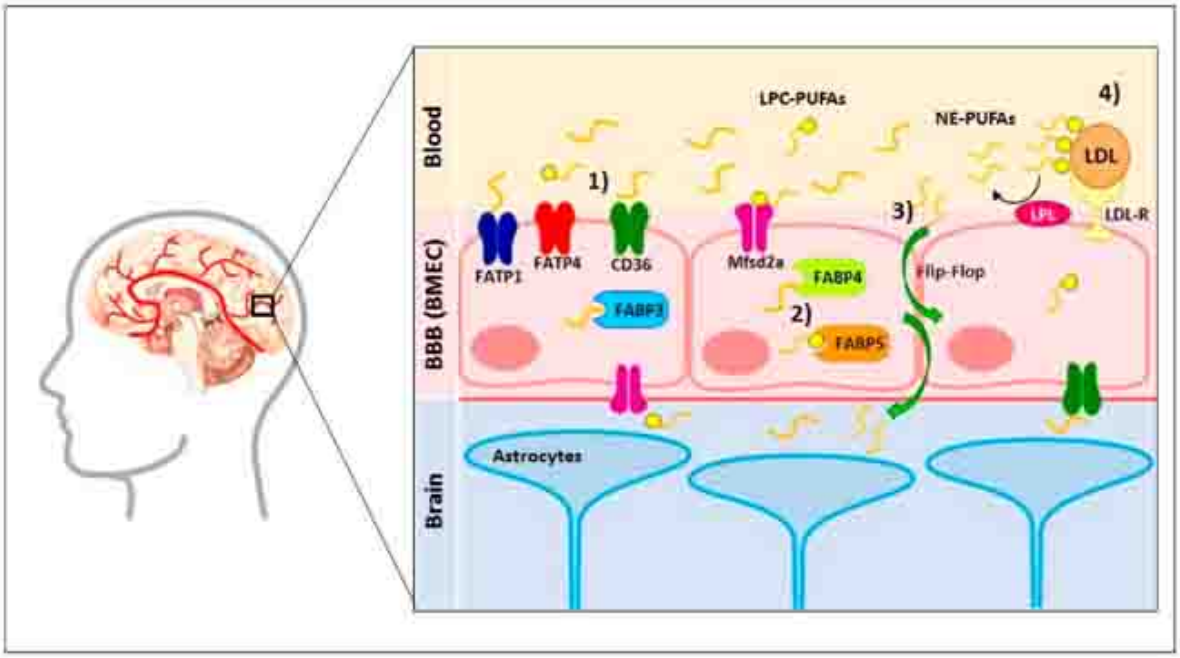

Fig. (3). Mechanistic insight into the role of PUFA-based nanocarriers in brain targeting across the BBB for treatment of GBM (The polyunsaturated fatty acid crosses the BBB mechanism, showing that PUFAs have the advantage to cross the BBB with the help of a non-fenestrated layer of cerebral microvascular endothelial cells (BMEC). There are four ways: (**1**) Membrane-localized fatty acid transports proteins (FATP1, FATP4, fatty acid translocase (FAT)/CD36, and Mfsd2a). (**2**) Cytosolic-localized fatty acid-binding proteins (FABP3, FABP4, and FABP5); this ligand-receptor union facilitates the brain fatty acid uptake and trafficking. FATP1 and FABP5 regulate the brain uptake of NE-DHA. Mfsd2a is the major contributor of LPC-DHA; the FATP-4, CD36, and FABP5 receptors promote the permeability of the LA. (**3**) With the help of passive diffusion, PUFAs can cross the BBB through a flip-flop mechanism. (**4**) Low-density lipoproteins (LDL) bind to the low-density lipoprotein receptor (LDL-R), and then lipoprotein lipases (LPL) liberate PUFAs by hydrolysis of ester bonds (LPC-PUFAs Lysophosphatidylcholine-Polyunsaturated fatty acids, NE-PUFAs Non-Esterified-Polyunsaturated fatty acids) (adapted from Ref. [157]). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

1.10. GLA Action on the Drug-resistance Cell of the Tumor

In the treatment of cancer, drug resistance is a significant hindrance. KB-ChR-8-5 cells remain unaffected by the cytotoxic action of vincristine compared to KB-3-1 cells, which are sensitive to this action. However, GLA could kill both vincristine-resistant and sensitive human cervical carcinoma cells [128, 144]. According to other studies, indomethacin, which is a CO inhibitor, and NDGA, an inhibitor of the synthesis of leukotriene were ineffective in blocking the cytotoxic action of GLA. GLA and other PUFAs enhanced free radical generation and lipid peroxide formulation to KB-3-1 and KB-ChR-8-5 cells. Moreover, antioxidants superoxide dismutase and vitamin E not only blocked their cytotoxic action but also inhibited the generation of superoxide anion and lipid peroxides [128, 144]. Therefore, studies have recommended that GLA can kill even drug-resistant tumor cells with the help of a free radical-dependent process.

1.11. GLA for Glioma

According to the various studies on glioma, GLA and other PUFAs established the anti-tumor effects [133, 136, 145]. In both *in vivo and in vitro* studies, GLA exerted cytotoxic action on glioma cells. According to the studies, GLA has the property to exert anticancer activity on glioma cells without harming the healthy neuronal cells and exhibit low or no neurotoxicity in both human and animal models [16, 136]. It has been observed that by increasing the generation of free radicals, GLA induces apoptosis of glioma cells [133]. According to the studies, the most effective concentration of GLA is 2 mM/l. However, when GLA at 1 ml/h concentration was infused in the animal glioma model, it exhibited preservation of neurons and the absence of inflammatory changes in surrounding brain tissue. This concentration of GLA, which produced significant tumor regression in the C6 implantation glioma model (0.05 mmole/day, 0.34 mmole over 7 days), was comparable to the GLA dose infused into human glioma (human gliomas at detection are 2-40 times the size of the rodent glioma tumors) using a similar infusion protocol (4 mmole/day, 28-40 mmole over 7-10 days) [16, 146]. Studies have shown that the intratumoral injection was more effective than oral and parenteral administration [147]. Findings of such types of studies have shown that various GLA methods, especially tumor cells, are needed to enhance the tumoricidal actions of GLA. In this regard, Fig. (**3**) illustrates various mechanistic insights into the role of PUFA-based nanocarriers in brain targeting across the BBB for the treatment of GBM.

1.12. Synergistic Effect of GLA

GLA has the ability not only to act as an anticancer agent, but for the other anticancer drugs, GLA enhances tumoricidal actions. Subsequently, in tumor cells, PUFAs improve the formation of free radicals and lipid peroxidation; it is predictable that both GLA and other drugs used for cancer treatment show synergistic tumoricidal action. GLA, EPA, doxorubicin, cis-platinum, and vincristine improved the cytotoxicity of the anticancer drugs to HeLa cells *in vitro* [148]. According to the drug uptake study, to alter their fluidity and permeability into the cancerous cell, they get joined and, as a result, increase drug acceptance by HeLa cells. With the help of this, intracellular concentration is enhanced. In the same way, as radiation-induced cell kill, GLA has been reported to increase the astrocytoma cells' sensitivity in rats [149]. It has been found that GLA may decrease tumor cells in glioma patients. Moreover, GLA given to breast cancer patients for six weeks in an amount of 20 mg/day, orally with tamoxifen, showed a faster response than tamoxifen [150]. These results of the phase II study showed that orally given GLA is beneficial in the treatment of ER+ breast cancer.

1.13. GLA Shows Cytoprotection to Healthy Cells

According to the experiment done on the animals (rats and dogs), GLA's infusion and injection into the brain parenchyma do not show any after effect, and for the healthy cell, GLA exhibits cytoprotective activity [17, 136]. In a study on GLA involving 15 normal rats, GLA infused at concentrations of 20 and 40 mM/1 was injected into the brain of the rats for 7 days [136]. The histological studies have shown tissue injury in a minimal area of brain tissue. These results recommended that due to the surgical treatment and infusion of GLA, the reactive damage was lesser compared to tumor infiltration. In a study involving three healthy dogs, GLA injection into the brain parenchyma was reported to be harmless [17].

GLA was injected into the dog's brain tissue through a cannula with 0.25mg saline under aseptic conditions for six days. Their brains were removed by sacrificing the animals on the seventh day. Computerized axial tomography (CT) was performed to visualize the changes in the attenuation values in the surrounding cerebral cortex where GLA was injected and compared to the left cerebral hemisphere. The histopathological studies have reported no side effects. No other abnormality was disclosed in the CT scans. Additionally, the results showed no sign of injury nor any adverse effects on the CNS function of the treated animals. Many studies have shown that GLA may essentially shield healthy tissues from cytotoxic actions [17].

Studies have shown that Benzo[a]pyrene (BP) given by oral route (75 mg/kg of body weight) and complete body gamma-radiation (250 rads, 1 Gy/min) significantly increase the polychromatic amount of RBC[151, 152]. When GLA is given one h after this drug or radiation intraperitoneally, the genetic injury is completely prevented [151-153]. To evaluate the fetus on the post-meiotic, meiotic, and pre-meiotic stages of spermatogenesis, the Dominant lethal test (DLT) is used. GLA produces Prostaglandin E1 for the rate reduction of dead implants. This study showed that GLA is not injurious to the fetus and, indeed, protects the fetus from damagingfactors [152].

1.14. GLA: Clinical Studies on Glioma

Encouraged by the GLA's study, *in vitro* and *in vivo* clinical studies on glioma/ anaplastic astrocytoma have been conducted. Intratumoral injection of GLA at a dose of 1 mg/day for 7-10 days showed tumor size reduction devoid of any acute side effects[16]. GLA was implanted (1mg) in the reservoir cavity of a cerebral catheter present under the galea's bone flap in the tumor bed suture, which was removed on the 7th postoperative day for pre-GLA therapy; a brain CT was taken with or without contrast. Under strict aseptic conditions, GLA (1mg) and sterile saline (2-3mg) were implanted in the cerebral reservoir every day.

For post-GLA therapy, a repeat CT scan was performed (with and without contrast) following ten days of GLA therapy. After completion of GLA therapy, radiotherapy was given to all the patients. All 15 patients tolerated the treatment well, and considering GLA therapy, no side effects or difficulties were observed. In the CT scan, a remarkable variation in tumor bed morphology was observed. Gliomas with high-density regions turned into less-density ones [17].

A cyst size enhancement, which is a symptom of tumor necrosis, and a midline shift reduction, were noted in all the patients. After the studies, three patients out of 15 died during 5 to 8 months. Total 12 patients did not indicate reappearance during the follow-up period, recommending that GLA is beneficial in human gliomas treatment. According to this study, the intratumoral injection of GLA did not show any significant side effects or toxicity [17].

In an open-label clinical study, patients with gliomas (grade 4) were treated with GLA intratumoral injection. In this study, nine patients were enrolled, and 1 mg of GLA per day for 7 consecutive days was given to them. Further radiotherapy or chemotherapy was not suitable for them because they had advanced gliomas. These patients died. Before starting GLA therapy, seven out of nine went through surgical procedures and radiotherapy. Over 11 months, one patient who underwent three cytoreductive surgeries was given GLA treatment as a one last salvage method. This patient's neurosurgical status did not improve significantly, but the radiologic images showed a significant reduction in the tumor after GLA therapy. CT and MRI (magnetic resonance image) scan analysis of all the patients [9] showed a response to GLA administration, reducing tumor tissues, peritumoral edema, and cystic areas. Moreover, no significant complication was observed in any patient [154].

CONCLUSION

According to the various studies discussed above, it is evident that tumor cell apoptosis is induced by GLA with little or no action on healthy cells. According to the multiple reviews on tumor cells, the GLA could block cytotoxic action with the help of antioxidants. A 2-3fold increase was observed in the number of lipid peroxides and free radicals in GLA-treated tumors without affecting the normal cell. It has been found that GLA-induced tumor cell apoptosis is a free radical-dependent process [12, 132]. Besides, due to augmented free radical generation, GLA reduced the antioxidant content, inhibited oncogenes RAS expression and Bcl-2, enriched P53 activity, and formation of lipid peroxides in tumor cells [135-137].

GLA could be beneficial in the management of drug-resistant tumors. GLA has the capability to protect against radiation and DNA damage, which is chemical-induced, increase the cytotoxic effect of drugs that are used in cancer treatment, and induce inverse drug resistance for a tumor cell. According to animal studies, GLA plays an active role in skin papilloma and does not permit chemical-induced hepatocarcinogenesis and ascitic tumors [129, 155]. Intravenous administration of lithium GLA to cancer patients was found to be relatively non-toxic [156]. GLA preserved the normal and healthy cells of the brain as well as the regression of tumors in the animal glioma model of animals. The three open-label clinical studies revealed that GLA intra-tumoral injection is useful in reducing the size of glioma and does not cause any significant side effects. However, more studies are required to evaluate the anti-cancer potential of GLA [16, 17].

According to specific requirements, the characteristic features of these nanoformulations can be manipulated. Utilizing the nanomedicine approaches, GLA is used in new formulations for various disease treatments with maximum benefit and less toxicity or side effect. So far, only limited studies have been performed to evaluate the *in vitro* or *in vivo* effects of nano encapsulated ω-3-PUFAs. A practical mechanistic approach should be implemented to compare how ω-3-PUFA nanoformulations enhance the therapeutic activity and control the disease's progression, recurrence, and disorder. All these preliminary investigations categorize suitable ω-3-PUFA nanoformulations that have anticipated optimum loading, targeting, and releasing capacity. The possible influence of nanoencapsulation of ω-3-PUFAs in therapeutic delivery is greatly promising, though some issues that prevent their widespread clinical use still need to be addressed. Therefore, developing such NDDS would improve patient compliance by increasing the potential risk-benefit ratio.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Louis, D.N.; Perry, A.; Reifenberger, G.; von Deimling, A.; Figarella-Branger, D.; Cavenee, W.K.; Ohgaki, H.; Wiestler, O.D.; Kleihues, P.; Ellison, D.W. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol.,* **2016**, *131*(6), 803-820. http://dx.doi.org/10.1007/s00401-016-1545-1 PMID: 27157931

[2] Vasilev, A.; Sofi, R.; Rahman, R.; Smith, S.J.; Teschemacher, A.G.; Kasparov, S. Using light for therapy of glioblastoma multiforme (GBM). *Brain Sci.,* **2020**, *10*(2), 75. http://dx.doi.org/10.3390/brainsci10020075 PMID: 32024010

[3] Inda, M.M.; Bonavia, R.; Seoane, J. Glioblastoma multiforme: a look inside its heterogeneous nature. *Cancers (Basel),* **2014**, *6*(1), 226-239. http://dx.doi.org/10.3390/cancers6010226 PMID: 24473088

[4] Amin, A.R.; Kucuk, O.; Khuri, F.R.; Shin, D.M. Perspectives for cancer prevention with natural compounds. *J. Clin. Oncol*., **2009**, *27*(16), 2712-2725. http://dx.doi.org/10.1200/JCO.2008.20.6235

[5] Adlercreutz, H.; Mazur, W. Phyto-oestrogens and Western diseases. *Ann. Med.,* **1997**, *29*(2), 95-120. http://dx.doi.org/10.3109/07853899709113696 PMID: 9187225

[6] Ahmad, I.U.; Forman, J.D.; Sarkar, F.H.; Hillman, G.G.; Heath, E.; Vaishampayan, U.; Cher, M.L.; Andic, F.; Rossi, P.J.; Kucuk, O. Soy isoflavones in conjunction with radiation therapy in patients with prostate cancer. *Nutr. Cancer,* **2010**, *62*(7), 996-1000. http://dx.doi.org/10.1080/01635581.2010.509839 PMID: 20924975

[7] Sandberg, C.J.; Altschuler, G.; Jeong, J.; Strømme, K.K.; Stangeland, B.; Murrell, W.; Grasmo-Wendler, U.H.; Myklebost, O.; Helseth, E.; Vik-Mo, E.O.; Hide, W.; Langmoen, I.A. Comparison of glioma stem cells to neural stem cells from the adult human brain identifies dysregulated Wnt-signaling and a fingerprint associated with clinical outcome. *Exp. Cell Res.,* **2013**, *319*(14), 2230-2243. http://dx.doi.org/10.1016/j.yexcr.2013.06.004 PMID: 23791939

[8] a) Vinogradov, S.; Wei, X. Cancer stem cells and drug resistance: the potential of nanomedicine. *Nanomedicine*., **2012**, *7*(4), 597-615. b) Puli S, Lai JC, Bhushan A. Inhibition of matrix degrading enzymes and invasion in human glioblastoma (U87MG) cells by isoflavones. *J. Neurooncol.,* **2006**, *79*(2), 135-142. PMID: 16598420

[9] a) Bapat, A.; Fishel, M.L.; Kelley, M.R. Going ape as an approach to cancer therapeutics. *Antioxidants & Redox Signaling*, **2009**; *11*(3), 651-667. b) Goodman M, Bostick RM, Kucuk O, Jones DP. Clinical trials of antioxidants as cancer prevention agents: past, present, and future. *Free Radic. Biol. Med.,* **2011**, *51*(5), 1068-1084. PMID: 21683786

[10] a) Singh-Gupta, V.; Joiner, M.C.; Runyan, L.; Yunker, C.K.; Sarkar, F.H.; Miller, S. Soy isoflavones augment radiation effect by inhibiting APE1/Ref-1 DNA repair activity in non-small cell lung cancer. *J. Thoracic Oncol*., **2011**, *6*(4), 688-698. b) Virk-Baker MK, Nagy TR, Barnes S. Role of phytoestrogens in cancer therapy. *Planta Med.,* **2010**, *76*(11), 1132-1142. PMID: 20597043

[11] Albino, A.P.; Juan, G.; Traganos, F.; Reinhart, L.; Connolly, J.; Rose, D.P.; Darzynkiewicz, Z. Cell cycle arrest and apoptosis of melanoma cells by docosahexaenoic acid: association with decreased pRb phosphorylation. *Cancer Res.,* **2000**, *60*(15), 4139-4145. PMID: 10945621

[12] Das, U.N. Tumoricidal action of *cis*-unsaturated fatty acids and their relationship to free radicals and lipid peroxidation. *Cancer Lett.,* **1991**, *56*(3), 235-243. http://dx.doi.org/10.1016/0304-3835(91)90008-6 PMID: 1850658

[13] Das, U.N. Gamma-linolenic acid, arachidonic acid, and eicosapentaenoic acid as potential anticancer drugs. *Nutrition,* **1990**, *6*(6), 429-434. PMID: 1966883

[14] Begin, M.; Das, U.; Ells, G. Cytotoxic effects of essential fatty acids (EFA) in mixed cultures of normal and malignant human cells. *Prog. Lipid Res.,* **1986**, *25*, 573-576. http://dx.doi.org/10.1016/0163-7827(86)90116-5

[15] a) Karmali, R.A.; Marsh, J.; Fuchs, C. Effect of omega-3 fatty acids on growth of a rat mammary tumor. *JNCI.,* **1984**, *73*(2), 457-61. b) El-Ela SHA, Prasse KW, Carroll R, Bunce OR. Effects of dietary primrose oil on mammary tumorigenesis induced by 7, 12-dimethylbenz (a) anthracene. *Lipids*, **1987**, *22*(12), 1041-4. c) Rose DP, Connolly JM. Antiangiogenicity of docosahexaenoic acid and its role in the suppression of breast cancer cell growth in nude mice. *Int. J. Oncol.,* **1999**, *15*(5), 1011-1016. PMID: 10536186

[16] Naidu, M.R.; Das, U.N.; Kishan, A. Intratumoral gamma-linoleic acid therapy of human gliomas. *Prostaglandins Leukot. Essent. Fatty Acids,* **1992**, *45*(3), 181-184. http://dx.doi.org/10.1016/0952-3278(92)90110-5 PMID: 1589443

[17] Das, U.N.; Prasad, V.V.; Reddy, D.R. Local application of γ-linolenic acid in the treatment of human gliomas. *Cancer Lett.,* **1995**, *94*(2), 147-155. http://dx.doi.org/10.1016/0304-3835(95)03844-M PMID: 7634242

[18] Bell, J.G.; Castell, J.D.; Tocher, D.R.; Macdonald, F.M.; Sargent, J.R. Effects of different dietary arachidonic acid: docosahexaenoic acid ratios on phospholipid fatty acid compositions and prostaglandin production in juvenile turbot (*Scophthalmus maximus*). *Fish Physiol. Biochem.,* **1995**, *14*(2), 139-151. http://dx.doi.org/10.1007/BF00002457 PMID: 24197361

[19] Mathers, L.; Bailey, M.J. Enzyme deletions and essential fatty acid metabolism in cultured cells. *J. Biol. Chem.,* **1975**, *250*(3), 1152-1153. PMID: 1120775

[20] Pourgholi, F.; Hajivalili, M.; Farhad, J.N.; Kafil, H.S.; Yousefi, M. Nanoparticles: Novel vehicles in treatment of glioblastoma. *Biomed. Pharmacother.,* **2016**, *77*, 98-107. http://dx.doi.org/10.1016/j.biopha.2015.12.014 PMID: 26796272

[21] Alifieris, C.; Trafalis, D.T. Glioblastoma multiforme: Pathogenesis and treatment. *Pharmacol. Ther.,* **2015**, *152*, 63-82. http://dx.doi.org/10.1016/j.pharmthera.2015.05.005 PMID: 25944528

[22] Roder, C.; Bisdas, S.; Ebner, F.H.; Honegger, J.; Naegele, T.; Ernemann, U.; Tatagiba, M. Maximizing the extent of resection and survival benefit of patients in glioblastoma surgery: high-field iMRI *versus* conventional and 5-ALA-assisted surgery. *Eur. J. Surg. Oncol.,* **2014**, *40*(3), 297-304. http://dx.doi.org/10.1016/j.ejso.2013.11.022 PMID: 24411704

[23] Urbańska, K.; Sokołowska, J.; Szmidt, M.; Sysa, P. Glioblastoma multiforme - an overview. *Contemp. Oncol. (Pozn.),* **2014**, *18*(5), 307-312. http://dx.doi.org/10.5114/wo.2014.40559 PMID: 25477751

[24] Kang, M-K.; Kang, S-K. Tumorigenesis of chemotherapeutic drug-resistant cancer stem-like cells in brain glioma. *Stem Cells Dev.,* **2007**, *16*(5), 837-847. http://dx.doi.org/10.1089/scd.2007.0006 PMID: 17999604

[25] Abbott, N.J. Blood-brain barrier structure and function and the challenges for CNS drug delivery. *J. Inherit. Metab. Dis.,* **2013**, *36*(3), 437-449. http://dx.doi.org/10.1007/s10545-013-9608-0 PMID: 23609350

[26] Miller, D.S. Regulation of ABC transporters blood-brain barrier: the good, the bad, and the ugly. In: *Advances in cancer research. 125*; Elsevier: Amterdem, **2015**, pp. 43-70. http://dx.doi.org/10.1016/bs.acr.2014.10.002

[27] Suzuki, C.; Jacobsson, H.; Hatschek, T.; Torkzad, M.R.; Bodén, K.; Eriksson-Alm, Y.; Berg, E.; Fujii, H.; Kubo, A.; Blomqvist, L. Radiologic measurements of tumor response to treatment: Practical approaches and limitations. *Radiographics,* **2008**, *28*(2), 329-344. http://dx.doi.org/10.1148/rg.282075068 PMID: 18349443

[28] Yan, Y.; Xu, Z.; Dai, S.; Qian, L.; Sun, L.; Gong, Z. Targeting autophagy to sensitive glioma to temozolomide treatment. *J. Exp. Clin. Cancer Res.,* **2016**, *35*(1), 23. http://dx.doi.org/10.1186/s13046-016-0303-5 PMID: 26830677

[29] a) Liau, L.M.; Ashkan, K.; Tran, D.D.; Campian, J.L.; Trusheim, J.E.; Cobbs, C.S. First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. *J. Translat. Med.,* **2018**, *16*(1), 142. b) Mujokoro B, Adabi M, Sadroddiny E, Adabi M, Khosravani M. Nanostructures mediated co-delivery of therapeutic agents for glioblastoma treatment: A review. *Mater. Sci. Eng. C,* **2016**, *69*, 1092-1102.

[30] Liang, J.; Lv, X.; Lu, C.; Ye, X.; Chen, X.; Fu, J. Prognostic factors of patients with gliomas-an analysis on 335 patients with glioblastoma and other forms of gliomas. *BMC Cancer,* **2020**, *20*(1), 1-7. http://dx.doi.org/10.1186/s12885-019-6511-6

[31] Brennan, C.W.; Verhaak, R.G.; McKenna, A.; Campos, B.; Noushmehr, H.; Salama, S.R.; Zheng, S.; Chakravarty, D.; Sanborn, J.Z.; Berman, S.H.; Beroukhim, R.; Bernard, B.; Wu, C.J.; Genovese, G.; Shmulevich, I.; Barnholtz-Sloan, J.; Zou, L.; Vegesna, R.; Shukla, S.A.; Ciriello, G.; Yung, W.K.; Zhang, W.; Sougnez, C.; Mikkelsen, T.; Aldape, K.; Bigner, D.D.; Van Meir, E.G.; Prados, M.; Sloan, A.; Black, K.L.; Eschbacher, J.; Finocchiaro, G.; Friedman, W.; Andrews, D.W.; Guha, A.; Iacocca, M.; O'Neill, B.P.; Foltz, G.; Myers, J.; Weisenberger, D.J.; Penny, R.; Kucherlapati, R.; Perou, C.M.; Hayes, D.N.; Gibbs, R.; Marra, M.; Mills, G.B.; Lander, E.; Spellman, P.; Wilson, R.; Sander, C.; Weinstein, J.; Meyerson, M.; Gabriel, S.; Laird, P.W.; Haussler, D.; Getz, G.; Chin, L. The somatic genomic landscape of glioblastoma. *Cell,* **2013**, *155*(2), 462-477. http://dx.doi.org/10.1016/j.cell.2013.09.034 PMID: 24120142

[32] a) Chang, S.M.; Wen, P.; Cloughesy, T.; Greenberg, H.; Schiff, D.; Conrad, C. Phase II study of CCI-779 in patients with recurrent glioblastoma multiforme. *Investigat. New Drugs*, **2005**, *23*(4), 357-61. b) Takeuchi H, Hashimoto N, Kitai R, Kubota T, Kikuta K-i. Proliferation of vascular smooth muscle cells in glioblastoma multiforme. *J. Neurosurg.,* **2010**, *113*(2), 218-224. PMID: 19929197

[33] Takano, S; Kimu, H; Tsuda, K; Osuka, S; Nakai, K; Yamamoto, T. Phase 2 study of CT-322, a targeted biologic inhibitor of VEGFR-2 based on a domain of human fibronectin, in recurrent glioblastoma. *Invest. New Drugs,* **2015**, *33*(1), 247-253. http://dx.doi.org/10.1007/s10637-014-0186-2 PMID: 25388940

[34] Thiessen, B.; Stewart, C.; Tsao, M.; Kamel-Reid, S.; Schaiquevich, P.; Mason, W.; Easaw, J.; Belanger, K.; Forsyth, P.; McIntosh, L.; Eisenhauer, E. A phase I/II trial of GW572016 (lapatinib) in recurrent glioblastoma multiforme: Clinical outcomes, pharmacokinetics and molecular correlation. *Cancer Chemother. Pharmacol.,* **2010**, *65*(2), 353-361. http://dx.doi.org/10.1007/s00280-009-1041-6 PMID: 19499221

[35] Aparicio-Blanco, J.; Sanz-Arriazu, L.; Lorenzoni, R.; Blanco-Prieto, M.J. Glioblastoma chemotherapeutic agents used in the clinical setting and in clinical trials: Nanomedicine approaches to improve their efficacy. *Int. J. Pharm.,* **2020**, *581*119283 http://dx.doi.org/10.1016/j.ijpharm.2020.119283 PMID: 32240807

[36] Zhao, M.; van Straten, D.; Broekman, M.L.D.; Préat, V.; Schiffelers, R.M. Nanocarrier-based drug combination therapy for glioblastoma. *Theranostics,* **2020**, *10*(3), 1355-1372. http://dx.doi.org/10.7150/thno.38147 PMID: 31938069

[37] Wang, Z.; Zhi, K.; Ding, Z.; Sun, Y.; Li, S.; Li, M., Pu, K.; Zou, J. Emergence in protein derived nanomedicine as anticancer therapeutics: More than a tour de force. *Semin. Cancer Biol.*, **2021**, *69*, 77-90.

[38] Linsley, C.S.; Wu, B.M. Recent advances in light-responsive on-demand drug-delivery systems. *Ther. Deliv.,* **2017**, *8*(2), 89-107. http://dx.doi.org/10.4155/tde-2016-0060 PMID: 28088880

[39] Ernsting, M.J.; Murakami, M.; Roy, A.; Li, S-D. Factors controlling the pharmacokinetics, biodistribution and intratumoral penetration of nanoparticles. *J. Control. Release,* **2013**, *172*(3), 782-794. http://dx.doi.org/10.1016/j.jconrel.2013.09.013 PMID: 24075927

[40] Duan, X.; Li, Y. Physicochemical characteristics of nanoparticles affect circulation, biodistribution, cellular internalization, and trafficking. *Small,* **2013**, *9*(9-10), 1521-1532. http://dx.doi.org/10.1002/smll.201201390 PMID: 23019091

[41] Choi, H.S.; Liu, W.; Misra, P.; Tanaka, E.; Zimmer, J.P.; Itty Ipe, B.; Bawendi, M.G.; Frangioni, J.V. Renal clearance of quantum dots. *Nat. Biotechnol.,* **2007**, *25*(10), 1165-1170. http://dx.doi.org/10.1038/nbt1340 PMID: 17891134

[42] Choi, J-S.; Cao, J.; Naeem, M.; Noh, J.; Hasan, N.; Choi, H-K.; Yoo, J.W. Size-controlled biodegradable nanoparticles: preparation and size-dependent cellular uptake and tumor cell growth inhibition. *Colloids Surf. B Biointerfaces,* **2014**, *122*, 545-551. http://dx.doi.org/10.1016/j.colsurfb.2014.07.030 PMID: 25108477

[43] a) Maeda, H. Macromolecular therapeutics in cancer treatment: The EPR effect and beyond. *J. Controlled Release,* **2012**, *164*(2), 138-44. b) Maeda H, Tsukigawa K, Fang J. A retrospective 30 years after discovery of the enhanced permeability and retention effect of solid tumors: Next-generation chemotherapeutics and photodynamic therapy-problems, solutions, and prospects. *Microcirculation,* **2016**, *23*(3), 173-182. http://dx.doi.org/10.1111/micc.12228 PMID: 26237291

[44] Stoehr, L.C.; Gonzalez, E.; Stampfl, A.; Casals, E.; Duschl, A.; Puntes, V.; Oostingh, G.J. Shape matters: effects of silver nanospheres and wires on human alveolar epithelial cells. *Part. Fibre Toxicol.,* **2011**, *8*(1), 36. http://dx.doi.org/10.1186/1743-8977-8-36 PMID: 22208550

[45] Xie, X.; Liao, J.; Shao, X.; Li, Q.; Lin, Y. The effect of shape on cellular uptake of gold nanoparticles in the forms of stars, rods, and triangles. *Sci. Rep.,* **2017**, *7*(1), 3827. http://dx.doi.org/10.1038/s41598-017-04229-z PMID: 28630477

[46] Wang, B.; Su, X.; Liang, J.; Yang, L.; Hu, Q.; Shan, X.; Wan, J.; Hu, Z. Synthesis of polymer-functionalized nanoscale graphene oxide with different surface charge and its cellular uptake, biosafety and immune responses in Raw264.7 macrophages. *Mater. Sci. Eng. C,* **2018**, *90*, 514-522. http://dx.doi.org/10.1016/j.msec.2018.04.096 PMID: 29853120

[47] Blanco, E.; Shen, H.; Ferrari, M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.,* **2015**, *33*(9), 941-951. http://dx.doi.org/10.1038/nbt.3330 PMID: 26348965

[48] Aftab, S.; Shah, A.; Nadhman, A.; Kurbanoglu, S.; Aysıl Ozkan, S.; Dionysiou, D.D.; Shukla, S.S.; Aminabhavi, T.M. Nanomedicine: An effective tool in cancer therapy. *Int. J. Pharm.,* **2018**, *540*(1-2), 132-149. http://dx.doi.org/10.1016/j.ijpharm.2018.02.007 PMID: 29427746

[49] Glaser, T.; Han, I.; Wu, L.; Zeng, X. Targeted nanotechnology in glioblastoma multiforme. *Front. Pharmacol.,* **2017**, *8*, 166. http://dx.doi.org/10.3389/fphar.2017.00166 PMID: 28408882

[50] Pardridge, W.M. Drug transport across the blood-brain barrier. *J. Cereb. Blood Flow Metab.,* **2012**, *32*(11), 1959-1972. http://dx.doi.org/10.1038/jcbfm.2012.126 PMID: 22929442

[51] Lee, S-W.; Kim, W.J.; Choi, Y.K.; Song, H.S.; Son, M.J.; Gelman, I.H.; Kim, Y.J.; Kim, K.W. SSeCKS regulates angiogenesis and tight junction formation in blood-brain barrier. *Nat. Med.,* **2003**, *9*(7), 900-906. http://dx.doi.org/10.1038/nm889 PMID: 12808449

[52] Shi, J.; Kantoff, P.W.; Wooster, R.; Farokhzad, O.C. Cancer nanomedicine: progress, challenges and opportunities. *Nat. Rev. Cancer,* **2017**, *17*(1), 20-37. http://dx.doi.org/10.1038/nrc.2016.108 PMID: 27834398

[53] Grinberg, S; Linder, C; Heldman, E Progress in lipid-based nanoparticles for cancer therapy. *Crit Rev. Oncog*., **2014**, *19*(3-4), 247-260. http://dx.doi.org/10.1615/CritRevOncog.2014011815

[54] Indoria, S.; Singh, V.; Hsieh, M.F. Recent advances in theranostic polymeric nanoparticles for cancer treatment: A review. *Int. J. Pharm.,* **2020**, *582*, 119314. http://dx.doi.org/10.1016/j.ijpharm.2020.119314 PMID: 32283197

[55] Wadajkar, A.S.; Dancy, J.G.; Hersh, D.S.; Anastasiadis, P.; Tran, N.L.; Woodworth, G.F.; Winkles, J.A.; Kim, A.J. Tumor-targeted nanotherapeutics: overcoming treatment barriers for glioblastoma. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.,* **2017**, *9*(4), e1439. http://dx.doi.org/10.1002/wnan.1439 PMID: 27813323

[56] Xing, H.; Hwang, K.; Lu, Y. Recent developments of liposomes as nanocarriers for theranostic applications. *Theranostics,* **2016**, *6*(9), 1336-1352. http://dx.doi.org/10.7150/thno.15464 PMID: 27375783

[57] a) Jhaveri, A.; Deshpande, P.; Pattni, B.; Torchilin, V. Transferrin-targeted, resveratrol-loaded liposomes for the treatment of glioblastoma. *J. Controlled Release*, **2018**, *277*, 89-101. b) Tapeinos, C.; Battaglini, M.; Ciofani, G. Advances in the design of solid lipid nanoparticles and nanostructured lipid carriers for targeting brain diseases. *J. Controlled Release*, **2017**, *264*, 306-32. c) Ortiz, R.; Cabeza, L.; Leiva, M.C.; Jimenez-Lopez, J.; Melguizo, C.; Prados, J. Nanomedical platform for drug delivery in cancer. *Curr. Organic Chem.*, **2017**, *21*(23), 2376-99. d) Sharma, G.; Modgil, A.; Sun, C.; Singh, J. Grafting of cell-penetrating peptide to receptor-targeted liposomes improves their transfection efficiency and transport

across blood-brain barrier model. *J. Pharm. Sci.,* **2012**, *101*(7), 2468-2478.
PMID: 22517732

[58] Ezzati Nazhad Dolatabadi, J.; Valizadeh, H.; Hamishehkar, H. Solid lipid nanoparticles as efficient drug and gene delivery systems: Recent breakthroughs. *Adv. Pharm. Bull.,* **2015**, *5*(2), 151-159.
http://dx.doi.org/10.15171/apb.2015.022 PMID: 26236652

[59] Huang, G.; Zhang, N.; Bi, X.; Dou, M. Solid lipid nanoparticles of temozolomide: Potential reduction of cardial and nephric toxicity. *Int. J. Pharm.,* **2008**, *355*(1-2), 314-320.
http://dx.doi.org/10.1016/j.ijpharm.2007.12.013 PMID: 18255242

[60] Kadari, A.; Pooja, D.; Gora, R.H.; Gudem, S.; Kolapalli, V.R.M.; Kulhari, H.; Sistla, R. Design of multifunctional peptide collaborated and docetaxel loaded lipid nanoparticles for antiglioma therapy. *Eur. J. Pharm. Biopharm.,* **2018**, *132*, 168-179.
http://dx.doi.org/10.1016/j.ejpb.2018.09.012 PMID: 30244167

[61] Ortiz, R.; Cabeza, L.; Perazzoli, G.; Jimenez-Lopez, J.; García-Pinel, B.; Melguizo, C.; Prados, J. Nanoformulations for glioblastoma multiforme: A new hope for treatment. *Future Med. Chem.,* **2019**, *11*(18), 2459-2480.
http://dx.doi.org/10.4155/fmc-2018-0521 PMID: 31544490

[62] Qu, J.; Zhang, L.; Chen, Z.; Mao, G.; Gao, Z.; Lai, X.; Zhu, X.; Zhu, J. Nanostructured lipid carriers, solid lipid nanoparticles, and polymeric nanoparticles: Which kind of drug delivery system is better for glioblastoma chemotherapy? *Drug Deliv.,* **2016**, *23*(9), 3408-3416.
http://dx.doi.org/10.1080/10717544.2016.1189465 PMID: 27181462

[63] Locatelli, E.; Naddaka, M.; Uboldi, C.; Loudos, G.; Fragogeorgi, E.; Molinari, V.; Pucci, A.; Tsotakos, T.; Psimadas, D.; Ponti, J.; Franchini, M.C. Targeted delivery of silver nanoparticles and alisertib: *In vitro* and *in vivo* synergistic effect against glioblastoma. *Nanomedicine (Lond.),* **2014**, *9*(6), 839-849.
http://dx.doi.org/10.2217/nnm.14.1 PMID: 24433240

[64] Yang, J; Lee, E; Ku, M; Huh, Y-M; Cheong, J-H Functional nanoplatforms for enhancement of chemotherapeutic index. *Anti-Cancer Agents Med. Chem.,* **2013**, *13*(2), 212-21.
http://dx.doi.org/10.2174/1871520611313020005

[65] Elzoghby, A.O.; Abd-Elwakil, M.M.; Abd-Elsalam, K.; Elsayed, M.T.; Hashem, Y.; Mohamed, O. Natural polymeric nanoparticles for brain-targeting: Implications on drug and gene delivery. *Curr. Pharm. Des.,* **2016**, *22*(22), 3305-3323.
http://dx.doi.org/10.2174/1381612822666160204120829 PMID: 26845323

[66] Raveendran, S.; Rochani, A.K.; Maekawa, T.; Kumar, D.S. Smart carriers and nanohealers: a nanomedical insight on natural polymers. *Materials (Basel),* **2017**, *10*(8), 929.
http://dx.doi.org/10.3390/ma10080929 PMID: 28796191

[67] Ruan, C.; Liu, L.; Lu, Y.; Zhang, Y.; He, X.; Chen, X.; Zhang, Y.; Chen, Q.; Guo, Q.; Sun, T.; Jiang, C. Substance P-modified human serum albumin nanoparticles loaded with paclitaxel for targeted therapy of glioma. *Acta Pharm. Sin. B,* **2018**, *8*(1), 85-96.
http://dx.doi.org/10.1016/j.apsb.2017.09.008 PMID: 29872625

[68] Singh, P.K.; Srivastava, A.K.; Dev, A.; Kaundal, B.; Choudhury, S.R.; Karmakar, S. 1, 3β-Glucan anchored, paclitaxel loaded chitosan nanocarrier endows enhanced hemocompatibility with efficient anti-glioblastoma stem cells therapy. *Carbohydr. Polym.,* **2018**, *180*, 365-375.
http://dx.doi.org/10.1016/j.carbpol.2017.10.030 PMID: 29103516

[69] Figueiredo, P.; Balasubramanian, V.; Shahbazi, M-A.; Correia, A.; Wu, D.; Palivan, C.G.; Hirvonen, J.T.; Santos, H.A. Angiopep2-functionalized polymersomes for targeted doxorubicin delivery to glioblastoma cells. *Int. J. Pharm.,* **2016**, *511*(2), 794-803.
http://dx.doi.org/10.1016/j.ijpharm.2016.07.066 PMID: 27484836

[70] Miura, Y.; Takenaka, T.; Toh, K.; Wu, S.; Nishihara, H.; Kano, M.R.; Ino, Y.; Nomoto, T.; Matsumoto, Y.; Koyama, H.; Cabral, H.; Nishiyama, N.; Kataoka, K. Cyclic RGD-linked polymeric micelles for targeted delivery of platinum anticancer drugs to glioblastoma through the blood-brain tumor barrier. *ACS Nano,* **2013**, *7*(10), 8583-8592.
http://dx.doi.org/10.1021/nn402662d PMID: 24028526

[71] Chen, X.; Tai, L.; Gao, J.; Qian, J.; Zhang, M.; Li, B.; Xie, C.; Lu, L.; Lu, W.; Lu, W. A stapled peptide antagonist of MDM2 carried by polymeric micelles sensitizes glioblastoma to temozolomide treatment through p53 activation. *J. Control. Release,* **2015**, *218*, 29-35.
http://dx.doi.org/10.1016/j.jconrel.2015.09.061 PMID: 26428461

[72] Quader, S.; Liu, X.; Chen, Y.; Mi, P.; Chida, T.; Ishii, T.; Miura, Y.; Nishiyama, N.; Cabral, H.; Kataoka, K. cRGD peptide-installed epirubicin-loaded polymeric micelles for effective targeted therapy against brain tumors. *J. Control. Release,* **2017**, *258*, 56-66.
http://dx.doi.org/10.1016/j.jconrel.2017.04.033 PMID: 28483513

[73] Li, J.; Yang, H.; Zhang, Y.; Jiang, X.; Guo, Y.; An, S.; Ma, H.; He, X.; Jiang, C. Choline derivate-modified doxorubicin loaded micelle for glioma therapy. *ACS Appl. Mater. Interfaces,* **2015**, *7*(38), 21589-21601.
http://dx.doi.org/10.1021/acsami.5b07045 PMID: 26356793

[74] Sarisozen, C.; Dhokai, S.; Tsikudo, E.G.; Luther, E.; Rachman, I.M.; Torchilin, V.P. Nanomedicine based curcumin and doxorubicin combination treatment of glioblastoma with scFv-targeted micelles: *In vitro* evaluation on 2D and 3D tumor models. *Eur. J. Pharm. Biopharm.,* **2016**, *108*, 54-67.
http://dx.doi.org/10.1016/j.ejpb.2016.08.013 PMID: 27569031

[75] Tan, X.; Kim, G.; Lee, D.; Oh, J.; Kim, M.; Piao, C.; Lee, J.; Lee, M.S.; Jeong, J.H.; Lee, M. A curcumin-loaded polymeric micelle as a carrier of a microRNA-21 antisense-oligonucleotide for enhanced anti-tumor effects in a glioblastoma animal model. *Biomater. Sci.,* **2018**, *6*(2), 407-417.
http://dx.doi.org/10.1039/C7BM01088E PMID: 29340361

[76] Sánchez-Purrà, M.; Ramos, V.; Petrenko, V.A.; Torchilin, V.P.; Borrós, S. Double-targeted polymersomes and liposomes for multiple barrier crossing. *Int. J. Pharm.,* **2016**, *511*(2), 946-956.
http://dx.doi.org/10.1016/j.ijpharm.2016.08.001 PMID: 27498281

[77] Kawamura, W.; Miura, Y.; Kokuryo, D.; Toh, K.; Yamada, N.; Nomoto, T.; Matsumoto, Y.; Sueyoshi, D.; Liu, X.; Aoki, I.; Kano, M.R.; Nishiyama, N.; Saga, T.; Kishimura, A.; Kataoka, K. Density-tunable conjugation of cyclic RGD ligands with polyion complex vesicles for the neovascular imaging of orthotopic glioblastomas. *Sci. Technol. Adv. Mater.,* **2015**, *16*(3), 035004.
http://dx.doi.org/10.1088/1468-6996/16/3/035004 PMID: 27877805

[78] Jiang, Y.; Yang, W.; Zhang, J.; Meng, F.; Zhong, Z. Protein toxin chaperoned by LRP‐1‐targeted virus‐mimicking vesicles induces high‐efficiency glioblastoma therapy *in vivo*. *Adv. Mater.,* **2018**, *30*(30), e1800316.
http://dx.doi.org/10.1002/adma.201800316 PMID: 29893017

[79] Abbasi, E.; Aval, S.F.; Akbarzadeh, A.; Milani, M.; Nasrabadi, H.T.; Joo, S.W.; Hanifehpour, Y.; Nejati-Koshki, K.; Pashaei-Asl, R. Dendrimers: synthesis, applications, and properties. *Nanoscale Res. Lett.,* **2014**, *9*(1), 247.
http://dx.doi.org/10.1186/1556-276X-9-247 PMID: 24994950

[80] Jiang, Y.; Lv, L.; Shi, H.; Hua, Y.; Lv, W.; Wang, X.; Xin, H.; Xu, Q. PEGylated Polyamidoamine dendrimer conjugated with tumor homing peptide as a potential targeted delivery system for glioma. *Colloids Surf. B Biointerfaces,* **2016**, *147*, 242-249.
http://dx.doi.org/10.1016/j.colsurfb.2016.08.002 PMID: 27518456

[81] Qiu, J.; Kong, L.; Cao, X.; Li, A.; Wei, P.; Wang, L.; Mignani, S.; Caminade, A.M.; Majoral, J.P.; Shi, X. Enhanced delivery of therapeutic siRNA into glioblastoma cells using dendrimer-entrapped gold nanoparticles conjugated with β-cyclodextrin. *Nanomaterials (Basel),* **2018**, *8*(3), 131.
http://dx.doi.org/10.3390/nano8030131 PMID: 29495429

[82] Liu, F-H.; Hou, C-Y.; Zhang, D.; Zhao, W-J.; Cong, Y.; Duan, Z.Y.; Qiao, Z.Y.; Wang, H. Enzyme-sensitive cytotoxic peptide-dendrimer conjugates enhance cell apoptosis and deep tumor penetration. *Biomater. Sci.,* **2018**, *6*(3), 604-613.
http://dx.doi.org/10.1039/C7BM01182B PMID: 29406549

[83] Chen, W; Zou, Y; Zhong, Z; Haag, R. *J. Control. Release,* **2016**, *239*, 159-168.
http://dx.doi.org/10.1016/j.jconrel.2016.08.029 PMID: 27569663

[84] Fan, L.; Yang, Q.; Tan, J.; Qiao, Y.; Wang, Q.; He, J.; Wu, H.; Zhang, Y. Dual loading miR-218 mimics and temozolomide using AuCOOH@FA-CS drug delivery system: Promising targeted antitumor drug delivery system with sequential release functions. *J. Exp. Clin. Cancer Res.,* **2015**, *34*(1), 106.
http://dx.doi.org/10.1186/s13046-015-0216-8 PMID: 26407971

[85] a) Fernández-Bertólez, N.; Costa, C.; Brandão, F.; Kiliç, G.; Duarte, J.A.; Teixeira, J.P. Toxicological assessment of silica-coated iron

oxide nanoparticles in human astrocytes. *Food Chem. Toxicol*., **2018**, *118*, 13-23. b) Lee, C.; Kim, G.R.; Yoon, J.; Kim, S.E.; Yoo, J.S.; Piao, Y. *In vivo* delineation of glioblastoma by targeting tumor-associated macrophages with near-infrared fluorescent silica coated iron oxide nanoparticles in orthotopic xenografts for surgical guidance. *Sci. Rep.,* **2018**, *8*(1), 1-12.
PMID: 29311619

[86] Meng, H.; Xue, M.; Xia, T.; Zhao, Y.L.; Tamanoi, F.; Stoddart, J.F.; Zink, J.I.; Nel, A.E. Autonomous *in vitro* anticancer drug release from mesoporous silica nanoparticles by pH-sensitive nanovalves. *J. Am. Chem. Soc.,* **2010**, *132*(36), 12690-12697.
http://dx.doi.org/10.1021/ja104501a PMID: 20718462

[87] Mo, J.; He, L.; Ma, B.; Chen, T. Tailoring particle size of mesoporous silica nanosystem to antagonize glioblastoma and overcome blood-brain barrier. *ACS Appl. Mater. Interfaces,* **2016**, *8*(11), 6811-6825.
http://dx.doi.org/10.1021/acsami.5b11730 PMID: 26911360

[88] Li, Z-Y.; Liu, Y.; Wang, X-Q.; Liu, L-H.; Hu, J-J.; Luo, G-F.; Chen, W.H.; Rong, L.; Zhang, X.Z. One-pot construction of functional mesoporous silica nanoparticles for the tumor-acidity-activated synergistic chemotherapy of glioblastoma. *ACS Appl. Mater. Interfaces,* **2013**, *5*(16), 7995-8001.
http://dx.doi.org/10.1021/am402082d PMID: 23869943

[89] Zhang, H.; Zhang, W.; Zhou, Y.; Jiang, Y.; Li, S. Dual functional mesoporous silicon nanoparticles enhance the radiosensitivity of VPA in glioblastoma. *Transl. Oncol.,* **2017**, *10*(2), 229-240.
http://dx.doi.org/10.1016/j.tranon.2016.12.011 PMID: 28193559

[90] Ahmadi Nasab, N.; Hassani Kumleh, H.; Beygzadeh, M.; Teimourian, S.; Kazemzad, M. Delivery of curcumin by a pH-responsive chitosan mesoporous silica nanoparticles for cancer treatment. *Artif. Cells Nanomed. Biotechnol.,* **2018**, *46*(1), 75-81.
http://dx.doi.org/10.1080/21691401.2017.1290648 PMID: 28278578

[91] Goel, S.; Chen, F.; Hong, H.; Valdovinos, H.F.; Hernandez, R.; Shi, S.; Barnhart, T.E.; Cai, W. $VEGF_{121}$-conjugated mesoporous silica nanoparticle: a tumor targeted drug delivery system. *ACS Appl. Mater. Interfaces,* **2014**, *6*(23), 21677-21685.
http://dx.doi.org/10.1021/am506849p PMID: 25353068

[92] Cheng, Y.; Morshed, R.; Cheng, S.H.; Tobias, A.; Auffinger, B.; Wainwright, D.A.; Zhang, L.; Yunis, C.; Han, Y.; Chen, C.T.; Lo, L.W.; Aboody, K.S.; Ahmed, A.U.; Lesniak, M.S. Nanoparticle-programmed self-destructive neural stem cells for glioblastoma targeting and therapy. *Small,* **2013**, *9*(24), 4123-4129.
http://dx.doi.org/10.1002/smll.201301111 PMID: 23873826

[93] a) Martincic, M.; Tobias, G. Filled carbon nanotubes in biomedical imaging and drug delivery. Expert opinion on drug delivery. **2015**, *12*(4), 563-581. b) Verma, J.; Lal, S.; Van Noorden, C.J. Nanoparticles for hyperthermic therapy: Synthesis strategies and applications in glioblastoma. *Int. J. Nanomedicine,* **2014**, *9*, 2863.

[94] Kafa, H.; Wang, J.T-W.; Rubio, N.; Venner, K.; Anderson, G.; Pach, E.; Ballesteros, B.; Preston, J.E.; Abbott, N.J.; Al-Jamal, K.T. The interaction of carbon nanotubes with an *in vitro* blood-brain barrier model and mouse brain *in vivo*. *Biomaterials,* **2015**, *53*, 437-452.
http://dx.doi.org/10.1016/j.biomaterials.2015.02.083 PMID: 25890741

[95] Moore, T.L.; Pitzer, J.E.; Podila, R.; Wang, X.; Lewis, R.L.; Grimes, S.W.; Wilson, J.R.; Skjervold, E.; Brown, J.M.; Rao, A.; Alexis, F. Multifunctional polymer-coated carbon nanotubes for safe drug delivery. *Part. Part. Syst. Charact.,* **2013**, *30*(4), 365-373.
http://dx.doi.org/10.1002/ppsc.201200145 PMID: 27642231

[96] Moore, T.L.; Grimes, S.W.; Lewis, R.L.; Alexis, F. Multilayered polymer-coated carbon nanotubes to deliver dasatinib. *Mol. Pharm.,* **2014**, *11*(1), 276-282.
http://dx.doi.org/10.1021/mp400448w PMID: 24294824

[97] Romano-Feinholz, S.; Salazar-Ramiro, A.; Muñoz-Sandoval, E.; Magaña-Maldonado, R.; Hernández Pedro, N.; Rangel López, E.; González Aguilar, A.; Sánchez García, A.; Sotelo, J.; Pérez de la Cruz, V.; Pineda, B. Cytotoxicity induced by carbon nanotubes in experimental malignant glioma. *Int. J. Nanomedicine,* **2017**, *12*, 6005-6026.
http://dx.doi.org/10.2147/IJN.S139004 PMID: 28860763

[98] Gholamine, B.; Karimi, I.; Salimi, A.; Mazdarani, P.; Becker, L.A. Neurobehavioral toxicity of carbon nanotubes in mice. *Toxicol. Ind. Health,* **2017**, *33*(4), 340-350.
http://dx.doi.org/10.1177/0748233716644381 PMID: 27230352

[99] Yang, K.; Feng, L.; Shi, X.; Liu, Z. Nano-graphene in biomedicine: Theranostic applications. *Chem. Soc. Rev.,* **2013**, *42*(2), 530-547.
http://dx.doi.org/10.1039/C2CS35342C PMID: 23059655

[100] Akhavan, O.; Ghaderi, E. Graphene nanomesh promises extremely efficient *in vivo* photothermal therapy. *Small,* **2013**, *9*(21), 3593-3601.
http://dx.doi.org/10.1002/smll.201203106 PMID: 23625739

[101] Yang, H-W.; Huang, C-Y.; Lin, C-W.; Liu, H-L.; Huang, C-W.; Liao, S-S.; Chen, P.Y.; Lu, Y.J.; Wei, K.C.; Ma, C.C. Gadolinium-functionalized nanographene oxide for combined drug and microRNA delivery and magnetic resonance imaging. *Biomaterials,* **2014**, *35*(24), 6534-6542.
http://dx.doi.org/10.1016/j.biomaterials.2014.04.057 PMID: 24811259

[102] Chowdhury, S.M.; Surhland, C.; Sanchez, Z.; Chaudhary, P.; Suresh Kumar, M.A.; Lee, S.; Peña, L.A.; Waring, M.; Sitharaman, B.; Naidu, M. Graphene nanoribbons as a drug delivery agent for lucanthone mediated therapy of glioblastoma multiforme. *Nanomedicine (Lond.),* **2015**, *11*(1), 109-118.
http://dx.doi.org/10.1016/j.nano.2014.08.001 PMID: 25131339

[103] Wei, Y.; Zhou, F.; Zhang, D.; Chen, Q.; Xing, D. A graphene oxide based smart drug delivery system for tumor mitochondria-targeting photodynamic therapy. *Nanoscale,* **2016**, *8*(6), 3530-3538.
http://dx.doi.org/10.1039/C5NR07785K PMID: 26799192

[104] Shim, G.; Kim, M-G.; Jin, H.; Kim, J.; Oh, Y-K. Claudin 4-targeted nanographene phototherapy using a *Clostridium perfringens* enterotoxin peptide-photosensitizer conjugate. *Acta Pharmacol. Sin.,* **2017**, *38*(6), 954-962.
http://dx.doi.org/10.1038/aps.2017.46 PMID: 28552914

[105] Wang, H.; Gu, W.; Xiao, N.; Ye, L.; Xu, Q. Chlorotoxin-conjugated graphene oxide for targeted delivery of an anticancer drug. *Int. J. Nanomedicine,* **2014**, *9*, 1433-1442.
PMID: 24672236

[106] Kargar, S.; Khoei, S.; Khoee, S.; Shirvalilou, S.; Mahdavi, S.R. Evaluation of the combined effect of NIR laser and ionizing radiation on cellular damages induced by IUdR-loaded PLGA-coated nano-graphene oxide. *Photodiagn. Photodyn. Ther.,* **2018**, *21*, 91-97.
http://dx.doi.org/10.1016/j.pdpdt.2017.11.007 PMID: 29155336

[107] Sawosz, E.; Jaworski, S.; Kutwin, M.; Vadalasetty, K.P.; Grodzik, M.; Wierzbicki, M.; Kurantowicz, N.; Strojny, B.; Hotowy, A.; Lipińska, L.; Jagiełło, J.; Chwalibog, A. Graphene functionalized with arginine decreases the development of glioblastoma multiforme tumor in a gene-dependent manner. *Int. J. Mol. Sci.,* **2015**, *16*(10), 25214-25233.
http://dx.doi.org/10.3390/ijms161025214 PMID: 26512645

[108] Song, J.; Yang, X.; Jacobson, O.; Lin, L.; Huang, P.; Niu, G.; Ma, Q.; Chen, X. Sequential drug release and enhanced photothermal and photoacoustic effect of hybrid reduced graphene oxide-loaded ultrasmall gold nanorod vesicles for cancer therapy. *ACS Nano,* **2015**, *9*(9), 9199-9209.
http://dx.doi.org/10.1021/acsnano.5b03804 PMID: 26308265

[109] a) Shao, J; Griffin, RJ; Galanzha, EI; Kim, J-W; Koonce, N; Webber, J Tunable, biodegradable gold nanoparticles as contrast agents for computed tomography and photoacoustic imaging. *Biomaterials*. **2016**, *102*, 87-97. b) Tong, L.; Wei, Q.; Wei, A.; Cheng, J.X. Gold nanorods as contrast agents for biological imaging: Optical properties, surface conjugation and photothermal effects. *Photochem. Photobiol.,* **2009**, *85*(1), 21-32.
PMID: 19161395

[110] Choi, J.; Yang, J.; Park, J.; Kim, E.; Suh, J.S.; Huh, Y.M. Specific near-IR absorption imaging of glioblastomas using integrin-targeting gold nanorods. *Adv. Funct. Mater.,* **2011**, *21*(6), 1082-1088.
http://dx.doi.org/10.1002/adfm.201002253

[111] Gonçalves, D.P.N.; Park, D.M.; Schmidt, T.L.; Werner, C. Modular peptide-functionalized gold nanorods for effective glioblastoma multicellular tumor spheroid targeting. *Biomater. Sci.,* **2018**, *6*(5), 1140-1146.
http://dx.doi.org/10.1039/C7BM01107E PMID: 29557480

[112] Locatelli, E.; Bost, W.; Fournelle, M.; Llop, J.; Gil, L.; Arena, F. Targeted polymeric nanoparticles containing gold nanorods: A therapeutic approach against glioblastoma. *J. Nanopart. Res.,* **2014**, *16*(3), 2304. http://dx.doi.org/10.1007/s11051-014-2304-7

[113] Neuwelt, E.A.; Várallyay, P.; Bagó, A.G.; Muldoon, L.L.; Nesbit, G.; Nixon, R. Imaging of iron oxide nanoparticles by MR and light microscopy in patients with malignant brain tumours. *Neuropathol. Appl. Neurobiol.,* **2004**, *30*(5), 456-471. http://dx.doi.org/10.1111/j.1365-2990.2004.00557.x PMID: 15488022

[114] Hong, H.; Shi, J.; Yang, Y.; Zhang, Y.; Engle, J.W.; Nickles, R.J.; Wang, X.; Cai, W. Cancer-targeted optical imaging with fluorescent zinc oxide nanowires. *Nano Lett.,* **2011**, *11*(9), 3744-3750. http://dx.doi.org/10.1021/nl201782m PMID: 21823599

[115] Arya, H.; Kaul, Z.; Wadhwa, R.; Taira, K.; Hirano, T.; Kaul, S.C. Quantum dots in bio-imaging: Revolution by the small. *Biochem. Biophys. Res. Commun.,* **2005**, *329*(4), 1173-1177. http://dx.doi.org/10.1016/j.bbrc.2005.02.043 PMID: 15766550

[116] Jackson, H.; Muhammad, O.; Daneshvar, H.; Nelms, J.; Popescu, A.; Vogelbaum, M.A.; Bruchez, M.; Toms, S.A. Quantum dots are phagocytized by macrophages and colocalize with experimental gliomas. *Neurosurgery,* **2007**, *60*(3), 524-529. http://dx.doi.org/10.1227/01.NEU.0000255334.95532.DD PMID: 17327798

[117] Weng, K.C.; Hashizume, R.; Noble, C.O.; Serwer, L.P.; Drummond, D.C.; Kirpotin, D.B.; Kuwabara, A.M.; Chao, L.X.; Chen, F.F.; James, C.D.; Park, J.W. Convection-enhanced delivery of targeted quantum dot-immunoliposome hybrid nanoparticles to intracranial brain tumor models. *Nanomedicine (Lond.),* **2013**, *8*(12), 1913-1925. http://dx.doi.org/10.2217/nnm.12.209 PMID: 23631502

[118] Lin, G.; Chen, T.; Zou, J.; Wang, Y.; Wang, X.; Li, J.; Huang, Q.; Fu, Z.; Zhao, Y.; Lin, M.C.; Xu, G.; Yong, K.T. Quantum dots-siRNA nanoplexes for gene silencing in central nervous system tumor cells. *Front. Pharmacol.,* **2017**, *8*, 182. http://dx.doi.org/10.3389/fphar.2017.00182 PMID: 28420995

[119] Manna, P.K.; Nickel, R.; Wroczynskyj, Y.; Yathindranath, V.; Li, J.; Liu, S.; Thliveris, J.A.; Klonisch, T.; Miller, D.W.; van Lierop, J. Simple, hackable, size-selective, amine-functionalized Fe-oxide nanoparticles for biomedical applications. *Langmuir,* **2018**, *34*(8), 2748-2757. http://dx.doi.org/10.1021/acs.langmuir.7b02822 PMID: 29376382

[120] Tudisco, C.; Cambria, M.; Giuffrida, A.; Sinatra, F.; Anfuso, C.; Lupo, G. Comparison between folic acid and gH625 peptide-based functionalization of Fe□O□ magnetic nanoparticles for enhanced cell internalization. *Nanoscale Res. Lett.,* **2018**, *13*(1), 1-10. http://dx.doi.org/10.1186/s11671-018-2459-8 PMID: 29299709

[121] Shen, Y.; Wu, C.; Uyeda, T.Q.P.; Plaza, G.R.; Liu, B.; Han, Y.; Lesniak, M.S.; Cheng, Y. Elongated nanoparticle aggregates in cancer cells for mechanical destruction with low frequency rotating magnetic field. *Theranostics,* **2017**, *7*(6), 1735-1748. http://dx.doi.org/10.7150/thno.18352 PMID: 28529648

[122] Stephen, Z.R.; Gebhart, R.N.; Jeon, M.; Blair, A.A.; Ellenbogen, R.G.; Silber, J.R.; Zhang, M. pH-sensitive O6-benzylguanosine polymer modified magnetic nanoparticles for treatment of glioblastomas. *Bioconjug. Chem.,* **2017**, *28*(1), 194-202. http://dx.doi.org/10.1021/acs.bioconjchem.6b00545 PMID: 27936607

[123] Richard, S; Saric, A; Boucher, M; Slomianny, C; Geffroy, F *Nanomedicine (Lond.),* **2015**, *11*(4), 825-833. PMID: 25652902

[124] a) Niedfeld, G.; De, M.T.; Mercuri, O.; Montoro, L. Microsomal fatty acid desaturation and elongation in a human lung carcinoma grown in nude mice. *Biochem. Internat*., **1988**, *16*(3), 413-420. b) Fermor, B.F.; Masters, J.R.; Wood, C.B.; Miller, J.; Apostolov, K.; Habib, N.A. Fatty acid composition of normal and malignant cells and cytotoxicity of stearic, oleic and sterculic acids *in vitro*. *European J. Cancer,* **1992**, *28*(6-7), 1143-7. c) Bardon, S.; Le, M.T.; Alessandri, J.M. Metabolic conversion and growth effects of n-6 and n-3 polyunsaturated fatty acids in the T47D breast cancer cell line. *Cancer letters*, **1996**, *99*(1), 51-8. d) Eynard, A.R. Potential of essential fatty acids as natural therapeutic products for human tumors. *Nutrition,* **2003**, *19*(4), 386. PMID: 12679177

[125] Leary, W; Robinson, K; Booyens, J; Dippenaar, N Some effects of gamma-linolenic acid on cultured human oesophageal carcinoma cells. *South African Med. J. Suid-Afrikaanse Tydskrif Vir Geneeskunde,* **1982**, *62*(19), 681-3.

[126] Bégin, M.E.; Das, U.N.; Ells, G.; Horrobin, D.F. Selective killing of human cancer cells by polyunsaturated fatty acids. *Prostaglandins Leukot. Med.,* **1985**, *19*(2), 177-186. http://dx.doi.org/10.1016/0262-1746(85)90084-8 PMID: 2864701

[127] Das, U.N. Essential fatty acids and their metabolites and cancer. *Nutrition,* **1999**, *15*(3), 239-240. PMID: 10198921

[128] Madhavi, N.; Das, U.N. Effect of n-6 and n-3 fatty acids on the survival of vincristine sensitive and resistant human cervical carcinoma cells *in vitro*. *Cancer Lett.,* **1994**, *84*(1), 31-41. http://dx.doi.org/10.1016/0304-3835(94)90355-7 PMID: 8076361

[129] Ramesh, G.; Das, U.N.; Koratkar, R.; Padma, M.; Sagar, P.S. Effect of essential fatty acids on tumor cells. *Nutrition,* **1992**, *8*(5), 343-347. PMID: 1330107

[130] a) Ramesh, G.; Das, U. Effect of dietary fat on diethylnitrosamine induced hepatocarcinogenesis in Wistar rats. *Cancer lett.*, **1995**, *95*(1-2), 237-245. b) Ramchurren, N.; Karmali, R. Effects of gammalinolenic and dihomo-gamma-linolenic acids on 7, 12-dimethylbenz (α) anthracene-induced mammary tumors in rats. *Prostaglandins Leukot. Essent. Fatty Acids,* **1995**, *53*(2), 95-101. PMID: 7480079

[131] Leaver, H.A.; Bell, H.S.; Rizzo, M.T.; Ironside, J.W.; Gregor, A.; Wharton, S.B.; Whittle, I.R. Antitumour and pro-apoptotic actions of highly unsaturated fatty acids in glioma. *Prostaglandins Leukot. Essent. Fatty Acids*, **2002**, *66*(1), 19-29. [PLEFA]. http://dx.doi.org/10.1054/plef.2001.0336 PMID: 12051954

[132] a) Das, U.; Begin, M.; Ells, G.; Huang, Y.; Horrobin, D. Polyunsaturated fatty acids augment free radical generation in tumor cells *in vitro*. *Biochem. Biophys. Res. Communicat.,* **1987**, *145*(1), 15-24. b) Colquhoun, A.; Schumacher, R.I. γ-Linolenic acid and eicosapentaenoic acid induce modifications in mitochondrial metabolism, reactive oxygen species generation, lipid peroxidation and apoptosis in Walker 256 rat carcinosarcoma cells. *Biochimica et Biophysica Acta*, **2001**, *1533*(3), 207-219.

[133] Leaver, H.A.; Williams, J.R.; Ironside, J.W.; Miller, E.P.; Gregor, A.; Su, B.H.; Prescott, R.J.; Whittle, I.R. Dynamics of reactive oxygen intermediate production in human glioma: n-6 essential fatty acid effects. *Eur. J. Clin. Invest.,* **1999**, *29*(3), 220-231. http://dx.doi.org/10.1046/j.1365-2362.1999.00418.x PMID: 10202379

[134] a) Ells, G.W.; Chisholm, K.A.; Simmons, V.A.; Horrobin, D.F. Vitamin E blocks the cytotoxic effect of γ-linolenic acid when administered as late as the time of onset of cell death-insight into the mechanism of fatty acid induced cytotoxicity. *Cancer Letters*, **1996**, *98*(2), 207-211. b) Chajès, V.; Sattler, W.; Stranzl, A.; Kostner, G.M. Influence of n-3 fatty acids on the growth of human breast cancer cells*in vitro*: Relationship to peroxides and vitamin-E. *Breast Cancer Res. Treat.,* **1995**, *34*(3), 199-212. PMID: 7579484

[135] Das, U.N. Essential fatty acids, lipid peroxidation and apoptosis. *Prostaglandins Leukot. Essent. Fatty Acids,* **1999**, *61*(3), 157-163. http://dx.doi.org/10.1054/plef.1999.0085 PMID: 10582655

[136] Leaver, H.A.; Wharton, S.B.; Bell, H.S.; Leaver-Yap, I.M.; Whittle, I.R. Highly unsaturated fatty acid induced tumour regression in glioma pharmacodynamics and bioavailability of gamma linolenic acid in an implantation glioma model: effects on tumour biomass, apoptosis and neuronal tissue histology. *Prostaglandins Leukot. Essent. Fatty Acids,* **2002**, *67*(5), 283-292. http://dx.doi.org/10.1054/plef.2002.0431 PMID: 12445487

[137] Das, U.N. From bench to the clinic: γ-linolenic acid therapy of human gliomas. *Prostaglandins Leukot. Essent. Fatty Acids,* **2004**, *70*(6), 539-552. http://dx.doi.org/10.1016/j.plefa.2003.12.001 PMID: 15120717

[138] Colquhoun, A. Gamma-linolenic acid alters the composition of mitochondrial membrane subfractions, decreases outer mitochondrial membrane binding of hexokinase and alters carnitine palmitoyltransferase I properties in the Walker 256 rat tumour. *Biochim. Biophys. Acta,* **2002**, *1583*(1), 74-84.

http://dx.doi.org/10.1016/S1388-1981(02)00162-2 PMID: 12069851

[139] Colquhoun, A.; Schumacher, R.I. Modifications in mitochondrial metabolism and ultrastructure and their relationship to tumour growth inhibition by gamma-linolenic acid. *Mol. Cell. Biochem.,* **2001**, *218*(1-2), 13-20. http://dx.doi.org/10.1023/A:1007243922393 PMID: 11330827

[140] Baron, J.A.; Cole, B.F.; Sandler, R.S.; Haile, R.W.; Ahnen, D.; Bresalier, R.; McKeown-Eyssen, G.; Summers, R.W.; Rothstein, R.; Burke, C.A.; Snover, D.C.; Church, T.R.; Allen, J.I.; Beach, M.; Beck, G.J.; Bond, J.H.; Byers, T.; Greenberg, E.R.; Mandel, J.S.; Marcon, N.; Mott, L.A.; Pearson, L.; Saibil, F.; van Stolk, R.U. A randomized trial of aspirin to prevent colorectal adenomas. *N. Engl. J. Med.,* **2003**, *348*(10), 891-899. http://dx.doi.org/10.1056/NEJMoa021735 PMID: 12621133

[141] Boudreau, M.D.; Sohn, K.H.; Rhee, S.H.; Lee, S.W.; Hunt, J.D.; Hwang, D.H. Suppression of tumor cell growth both in nude mice and in culture by n-3 polyunsaturated fatty acids: mediation through cyclooxygenase-independent pathways. *Cancer Res.,* **2001**, *61*(4), 1386-1391. PMID: 11245439

[142] Bégin, M.E.; Ells, G.; Das, U.N.; Horrobin, D.F. Differential killing of human carcinoma cells supplemented with n-3 and n-6 polyunsaturated fatty acids. *J. Natl. Cancer Inst.,* **1986**, *77*(5), 1053-1062. PMID: 3464797

[143] a) Das, U.N. Tumoricidal and anti-angiogenic actions of gamma-linolenic acid and its derivatives. *Curr. Pharmaceut. Biotechnol.,* **2006**, *7*(6), 457-466. http://dx.doi.org/10.2174/138920106779116892
b) Sagar, PS; Das, U; Koratkar, R Cytotoxic action of *cis*-unsaturated fatty acids on human cervical carcinoma (HeLa) cells: Relationship to free radicals and lipid peroxidation and its modulation by calmodulin antagonists. *Cancer lett.,* **1992**, *63*(3), 189-98.
(c) Das, U. Selective enhancement of free radicals by *cis*-unsaturated fatty acids in tumor cells as a strategy to kill tumor cells *in vitro* and *in vivo*. In: *Biological Oxidation Systems*, Reddy, C.C.; Madyastha, K.; Hamilton, A.K. Eds., Vol. 2, Academic Press: New York, **1990**, pp. 607-634.

[144] Madhavi, N.; Das, U. Reversal of KB-3-1 and KB-ChR-8-5 tumour cell drug-resistance by *cis*-unsaturated fatty acids *in vitro*. *Med. Sci. Res.,* **1994**, *22*(10), 689-692.

[145] a) Bell, H.S.; Wharton, S.B.; Leaver, H.A.; Whittle, I.R. Effects of N-6 essential fatty acids on glioma invasion and growth: experimental studies with glioma spheroids in collagen gels. *J. Neurosurg.,* **1999**, *91*(6), 989-96. b) Leaver, H.; Thomson, D.; Williams, J.; Bell, H.; Whittle, I. Essential fatty acid induced tumour regression in experimental glioma. *Br. J. Neurosurg.,* **1999**, *13*, 108.

[146] Das, U. Tumouricidal actions of gamma-linolenic acid with particular reference to the therapy of human gliomas. *Med. Sci. Res.,* **1995**, *23*(8), 507-513.

[147] Ravichandran, D.; Cooper, A.; Johnson, C.D. Effect of lithium γ-linolenate on the growth of experimental human pancreatic carcinoma. *Br. J. Surg.,* **1998**, *85*(9), 1201-1205. http://dx.doi.org/10.1046/j.1365-2168.1998.00838.x PMID: 9752859

[148] Sangeetha Sagar, P.; Das, U. Gamma-linolenic acid and eicosapentaenoic acid ptentiate the cytotoxicity of anti-cancer drugs on human cervical carcinoma (HeLa) cells *in vitro*. *Med. Sci. Res.,* **1993**, *21*(12), 457-459.

[149] Vartak, S.; Robbins, M.E.; Spector, A.A. Polyunsaturated fatty acids increase the sensitivity of 36B10 rat astrocytoma cells to radiation-induced cell kill. *Lipids,* **1997**, *32*(3), 283-292. http://dx.doi.org/10.1007/s11745-997-0035-y PMID: 9076665

[150] Kenny, F.S.; Pinder, S.E.; Ellis, I.O.; Gee, J.M.; Nicholson, R.I.; Bryce, R.P.; Robertson, J.F. Gamma linolenic acid with tamoxifen as primary therapy in breast cancer. *Int. J. Cancer,* **2000**, *85*(5), 643-648. http://dx.doi.org/10.1002/(SICI)1097-0215(20000301)85:5<643::AID-IJC8>3.0.CO;2-Z PMID: 10699943

[151] Das, U.N.; Ramadevi, G.; Rao, K.P.; Rao, M.S. Prostaglandins and their precursors can modify genetic damage-induced by gamma-radiation and benzo(a)pyrene. *Prostaglandins,* **1985**, *29*(6), 911-920. http://dx.doi.org/10.1016/0090-6980(85)90216-3 PMID: 2994175

[152] Das, U.N.; Ramadevi, G.; Rao, K.P.; Rao, M.S. Prostaglandins can modify gamma-radiation and chemical induced cytotoxicity and genetic damage *in vitro* and *in vivo*. *Prostaglandins,* **1989**, *38*(6), 689-716. http://dx.doi.org/10.1016/0090-6980(89)90050-6 PMID: 2633211

[153] Das, U.; Devi, G.R.; Rao, K.; Rao, M. Benzo (a) pyrene and gamma-radiation-induced genetic damage in mice can be prevented by gamma-linolenic acid but not by arachidonic acid. *Nutr. Res.,* **1985**, *5*(1), 101-105. http://dx.doi.org/10.1016/S0271-5317(85)80023-3

[154] Bakshi, A.; Mukherjee, D.; Bakshi, A.; Banerji, A.K.; Das, U.N. γ-linolenic acid therapy of human gliomas. *Nutrition,* **2003**, *19*(4), 305-309. http://dx.doi.org/10.1016/S0899-9007(02)00862-6 PMID: 12679162

[155] Ramesh, G.; Das, U.N. Effect of free fatty acids on two-stage skin carcinogenesis in mice. *Cancer Lett.,* **1996**, *100*(1-2), 199-209. http://dx.doi.org/10.1016/0304-3835(95)04095-1 PMID: 8620442

[156] Fearon, K.; Falconer, J.; Ross, J.; Carter, D.; Hunter, J.; Reynolds, P. An open-label phase I/II dose escalation study of the treatment of pancreatic cancer using lithium gammalinolenate. *Anticancer Res*., **1996**, *16*(2), 867-74. b) Johnson, C.; Puntis, M.; Davidson, N.; Todd, S.; Bryce, R. Randomized, dose-finding phase III study of lithium gamolenate in patients with advanced pancreatic adenocarcinoma. *Br. J. Surg.,* **2001**, *88*(5), 662-668. PMID: 11350436

[157] Montecillo-Aguado, M.; Tirado-Rodriguez, B.; Tong, Z.; Vega, O.M.; Morales-Martínez, M.; Abkenari, S.; Pedraza-Chaverri, J.; Huerta-Yepez, S. Importance of the role of ω-3 and ω-6 polyunsaturated fatty acids in the progression of brain cancer. *Brain Sci*., **2020**, *10*(6), 381-399. http://dx.doi.org/10.3390/brainsci10060381 PMID: 32560280; PMCID: PMC7349634