

# Nanotechnology-based cancer chemoprevention in glioblastoma

Aima Adylova<sup>1</sup>, Gulnara Kapanova<sup>2</sup>, Zaire Datkhayeva<sup>3</sup>, Karlygash Raganina<sup>4</sup>, Gulnur Tanbayeva<sup>2</sup>, Kaini Baigonova<sup>2</sup>

<sup>1</sup>Guangdong Key Laboratory for Genome Stability & Disease Prevention and Carson International Cancer Center, Marshall Laboratory of Biomedical Engineering, Shenzhen University Medical School, Guangdong, Shenzhen, China, <sup>2</sup>Al-Farabi Kazakh National University, Kazakhstan, <sup>3</sup>Department of Obstetrics and Gynaecology, Kazakh National Medical University, Kazakhstan, <sup>4</sup>Department of Pharmaceutical Technology, Asfendiyarov Kazakh National Medical University, Kazakhstan

*Folia Neuropathol* 2023; 61 (3): 235-241

DOI: <https://doi.org/10.5114/fn.2023.126886>

## Abstract

*Brain tumours are heterogeneous and are classified comprehensively into molecular subtypes based on genetic alterations. Glioblastoma rapid progression, drug resistance, and recurrence have been scientifically linked to several factors, including its rapid growth rate, loss of apoptosis, pro-survival signalling, molecular heterogeneities and hallmark features to infiltrate vital brain structures. Because of the growing demand for design and development of delivery systems to overcome the existing limitations with the current therapeutic strategies, researchers are exploiting multifaceted aspects of nanotechnology to improve delivery of the drug payload. Firstly, nanotechnology procedures can improve the drug delivery methods with the help of nanoparticles (NPs) based nanovectors that can efficiently cross blood-brain barrier. Secondly, NPs also improve the cellular uptake of the drug as they can efficiently bind with the cell surface. Thirdly, NPs make the delivery of siRNAs and peptides possible, which can suppress the resistance of glioblastoma against TMZ or other chemo-preventive drugs. Fourthly, the use of metal NPs increases the efficiency of scanning or magnetic resonance imaging (MRI) procedures as they can produce contrasts in it. Lastly, NPs make it possible to use highly targeted co-administered strategies like chemoprevention and near infrared (NIR) or radiotherapy (RT). Hence, nanotechnology offers several promising solutions against glioblastoma by countering it on many fronts.*

**Key words:** glioblastoma, malignant, temozolomide, nanoparticles, siRNAs.

## Introduction

Rapidly emerging preclinical and clinical studies have refined our understanding and highlighted the regulatory role of disease-associated heterogeneities. Cell signalling molecules orchestrated critical steps in cancer progression, genetic/epigenetic inactivation and apoptosis [3-10]. Based on the findings drawn from the insights gained by comprehensive investigation of a wide variety of cancers, researchers have witnessed landmark discoveries in molecular oncology. The state-of-the-art large-scale genetic, genomic and epigenomic profiling studies have decoded an unprecedented

wealth of novel data and provided better mechanistic insights into carcinogenesis and metastasis [18,19]. Scientists have explored wide ranging mechanisms including epigenetic modifications, apoptosis and how immunological responses play a role in regulation of cancer [23,28,31,36,39,40].

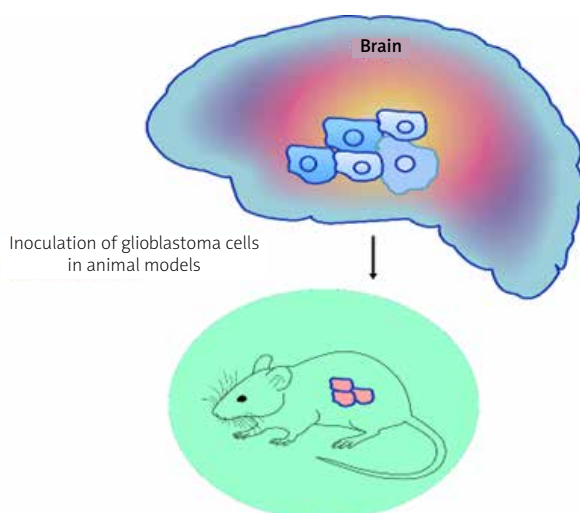
Glioblastomas have been the subject of increasingly rigorous research over the past two decades that has led to greater than ever broadening of our understanding about their basic biology and pathogenesis [21,24,27,34,35]. Genetic signatures have already been reported to play a central role in the pathogenesis of glioblastomas [29,32].

## Communicating author:

Ima Adylova, Guangdong Key Laboratory for Genome Stability & Disease Prevention and Carson International Cancer Center, Marshall Laboratory of Biomedical Engineering, Shenzhen University Medical School, Guangdong, 518060, Shenzhen, China, e-mail: [ima\\_gem@hotmail.co.uk](mailto:ima_gem@hotmail.co.uk)

These stumbling blocks are currently being addressed through the use of cutting-edge basic research works driven by next-generation technologies, including delivery of chemotherapeutic drugs and various antisense oligonucleotides [2,16,17]. Stimulation of the significant cross-disciplinary interest from the basic biomedical research and engineering fields has generated encouraging results. Recent scientific advancements have helped us in the identification of experimental approaches to overcome the outstanding challenges that presently limit delivery of the chemotherapeutics and antisense oligonucleotides in glioblastoma (GB).

Glioblastoma is a glial cell derived malignant brain tumour with a median survival rate of around 15 months [27]. Glioblastoma is usually characterized by a high heterogeneity at both cellular and molecular levels, high cell diffusion, dissemination, and vascularization. Glioblastoma can be further subdivided into different subtypes with proneural and mesenchymal being among the major ones. The prognosis for proneural subtype is not as challenging as compared to the mesenchymal subtype. Oncogenic transcription factor signal transducer and activator of transcription 3 (Stat3) has been reported to be upregulated in several classes of GB, especially the mesenchymal subtype [25]. Stat3 is known to upregulate the expression of several other oncogenes, making GB one of the most aggressive tumours of the brain. Glioma-associated-human mesenchymal stem cells (GA-hMSCs) played a role in tumorigenesis. The survival rates of rodent models that were implanted with tumour-initiating glioma stem cells cocultured with GA-hMSC were found to be significantly and critically reduced. Chemotherapy and radiotherapy



**Fig. 1.** Inoculation of glioblastoma-initiating cells in animal models for evaluation of anticancer agents.

are regarded as the first-line therapeutics against GB. For instance, chemotherapy with temozolomide (TMZ) and radiotherapy after surgical removal of the tumour mass is still the standard procedure available against GB. Encapsulation of temozolomide in a p-sulphonatocalix[4]arene (Calix) nanocapsule was found to be highly effective. Intracranial GBM xenograft models were intraperitoneally injected with TMZ@Calix complexes and there was an evident shrinkage of tumours [30]. TMZ is also known to possess some unwanted characteristics that highly limit its clinical applications. Due to a short half-life of 1.8 hours of TMZ along with its rapid removal from the body, a large quantity of the drug even fails to reach the site of tumour [35]. Administering larger quantities of TMZ could be a way forward, but it also increases chances of side effects. In 2009, Food and Drug Administration (FDA) approved an anti-VEGF (vascular endothelial growth factor) drug, named bevacizumab (Avastin®) against GB. However, this drug also has a low efficacy owing to difficulties in crossing blood-brain barrier (BBB), and off-site targeting. Hence, there is a need to look for the alternative drugs and drug delivery methods against GB. Using nanoparticles for drug delivery against GB have been found to be effective in several animal models. There are several advantages of using nanoparticles for drug delivery. Firstly, they ensure delivery to specific sites thereby reducing off-site targeting. Secondly, the specificity of nanoparticles reduces the number of doses required to achieve a required result. Thirdly, a direct delivery of nanoparticles is also possible, bypassing BBB. Lastly, the half-life of the drug is also increased once coated with a protective layer of nanoparticles. Bevacizumab-loaded poly (D, L-lactic-co-glycolic acid) nanoparticles (PLGA-NP) were administered intranasally to CD-1 animal models for the evaluation of pharmacodynamic and pharmacokinetic properties. Bevacizumab-loaded PLGA nanoparticles provided a higher availability of bevacizumab to the brain and efficiently inhibited tumour growth [33]. Nanoparticles developed against GB usually vary in a number of ways including composition, mechanism of action, mode of administration, and the type of drug they are carrying.

In this mini-review, we have summarized some of the cutting-edge research works which provided significant and translatable results about nanoparticle-based chemoprevention therapies against GB. In this regard, we will first discuss compositions and characteristics of nano-therapies, their mode of administration in animal models, and the mechanism of action against GB.

### Doxorubicin-loaded nanomicelles

Doxorubicin (DOX) is a well-known chemotherapy drug which is used against a number of cancers. RVG-29,

a 29-amino-acid polypeptide derived from rabies virus glycoprotein (RVG) has been reported to demonstrate brain-targeting capacity across the BBBs. Strategically, the size of RVG-29 was reduced for the generation of a 15-amino-acid polypeptide (RVG-15). Importantly, brain-targeting activity of RVG-15 across the blood-brain barrier remained intact. Doxorubicin-loaded polymeric micelles (DOX-RVG-15-PMs) penetrated the tumour tissues and released doxorubicin for the inhibition of tumour growth in mice stereotactically seeded with C6-luc cells into the target sites. DOX-RVG-15-PMs inhibited metastatic spread of primary cancer cells to the lungs and liver tissues of mice [12].

Targeted inhibition of the cancer-causing genes is indeed an exciting area of research. Protection of siRNAs from degradation not only promoted cellular uptake but also enhanced gene silencing efficiencies of siRNAs. Essentially, PEGylated cyclodextrin (CD)-based nanoparticles tagged with central nervous system (CNS)-targeting peptides derived from specific glycoprotein of the rabies virus have gained noteworthy attention [11].

### Catechol-based prodrug (Pt(IV)) with polymer nanoparticles

Researchers have tested catechol-based nanoparticles containing complex 1 (Pt(IV) prodrug) as an effective therapy against GB. The efficacy of these nanoparticles was compared with cisplatin, a well-known chemotherapeutic drug. Cisplatin is used against several types of cancers including breast, cervical, ovarian, testicular, neck and head cancer. It is generally used as a second-line therapy against cancer [22]. Researchers developed complex 1 containing iron nanoparticles by dissolving and processing a solution of complex 1, 96% ethanol, and iron acetate ( $\text{Fe}(\text{OAc})_2$ ). Analysis based on drug release profiling and cytotoxicity assays indicate that complex 1 nanoparticles had a higher ability to bypass BBB and cellular uptake as compared to cisplatin, a standard chemotherapeutic drug. Complex 1 nanoparticle treatment starting from day 6 of tumour development showed significantly better results as it managed to decrease the growth of tumour volume as compared to the standard. Another important reported aspect of the study was that tumour relapse was not found in the treated mice. Intranasally-administered drugs are absorbed rapidly through rich vasculatures in the submucosa, thus displaying a faster onset of action whereas reducing the toxic side effects. Intranasal administration of catechol-based nanostructured co-ordination polymers loaded with platinum (IV) prodrugs have remarkable anti-cancer effects. The orthotopic glioblastoma tumour-bearing animal models were experimentally generated by stereotactic injections of glioblastoma GL261 cells into C57BL/6J

striatum. Pt(IV)-based nanoparticles significantly inhibited tumour growth in GL261 orthotopic models [22]. The research team also suggested that balanced combinations of schedule and dose of administration should be optimized to obtain better response, both in pre-clinical and clinical studies.

### Biomimetic nanoparticles with Bcl-2/Bcl-xl and Mcl-1 inhibitors

Researchers also designed and tested the cancer targeting ability of biomimetic nanomedicines.

Researchers developed acetal-grafted dextran ( $\alpha$ -dextran) nanoparticles with Bcl2/Bcl-xl and Mcl-1 inhibitors. The Bcl-2 protein family, also known as anti-apoptosis proteins, contains several proteins that regulate mitochondrial pathways of apoptosis. These proteins have been found to impart anti-apoptosis properties in GB cells as they are usually overexpressed. Resultantly, GB cells do not respond to several apoptosis-inducing drugs. Therefore, targeting anti-apoptosis proteins can tilt the abnormal balance in favour of pro-apoptosis proteins like Bax and Bak. Researchers used ABT-263 (ABT) and A12, small molecule inhibitors of the Bcl2 family. ABT is already being used as an anti-cancer agent against several cancer types. ABT is known to mimic BH3-only proteins, and binds to anti-apoptosis proteins. However, ABT lacks the ability to inhibit Mcl-1. Therefore, the second inhibitor, A12, was also used in the nanoparticles. Therefore, based on the apolipoprotein E (ApoE) peptide-decorated red blood cell membrane and pH-sensitive dextran nanoparticles, researchers have experimentally investigated the targeted delivery of A12 and ABT to the glioblastoma cells. ApoE peptide has a superior binding affinity for different LDL family receptors (LRP1, LRP2, LDLR) overexpressed on the surface of endothelial cells of the BBB and GB cells, thus offering potent and advantageous tumour-targeting capability as compared to angioprep-2 for clinical applications. Firstly, both ABT and A12 were first loaded with  $\alpha$ -dextran to form nanoparticles. Secondly, the nanoparticles were further coated with a targeting ligand ApoE peptide-functionalized RBCm (ApoE-RBCm, AM). The resulting nanoparticles exhibited excellent BBB penetration and cellular uptake. Their anti-cancer effect was also analysed using U251-TR and CSC-2 cell lines, where the nanoparticles managed to induce apoptosis in a large proportion of tumour cells. Importantly, mice treated with AM@NP(ABT/A12) demonstrated considerable retardation of tumours as compared to those mice treated with nanomedicines loaded with ABT or A12 or free ABT/A12 mixtures. There was no noticeable loss of the body weight during treatment with AM@NP(ABT/A12) [15]. These findings clearly indicated that the biomimetic nanomedicines efficiently inhibited glioblastoma growth without exerting adverse side effects.

## Temozolomide-conjugated gold nanoparticle with photothermal therapy

A series of research works have highlighted a tumour inhibitory role of gold nanoparticles (GNPs) conjugated with TMZ. As mentioned earlier in this article, TMZ is a standard chemo-preventive drug used against GB. However, a large number of GB cases (ranging from 65% to 75%) exhibit resistance against TMZ treatment. Therefore, TMZ is quite often conjugated with other inhibitors and drugs to make it more effective. In this regard, researchers developed TMZ conjugated GNPs with anti-EphA3 (anti-EphA3-TMZ@GNPs). Anti-EphA3 is an antibody that selectively binds with tumour cells on ephrin receptor A3. EphA3 is generally overexpressed in several cancers and is involved in tumour progression. Studies indicate that EphA3 is involved in angiogenesis. Once administered in a host or added in a cell culture, anti-EphA3-TMZ@GNPs can be subjected to Plasma Photothermal Therapy (PPTT) to form GNPs-PPTT. Nanoparticles can absorb near infrared (NIR) light and increase their temperature. Hence, NIR can be specifically targeted at tumour cells, making highly targeted chemotherapy possible. During *in vitro* analysis, it was found that the NIR can increase the apoptosis capacity of anti-EphA3-TMZ@GNPs from 9.8% of cells to more than 65% of cells (GNPs-PPTT).

Anti-EphA3-TMZ@GNPs were also subjected to *in vivo* analysis in model mice. Mice were divided into four groups, and were administered with saline, TMZ, TMZ@GNPs, and anti-EphA3-TMZ@GNPs, respectively, in the tail vein. Furthermore, groups with TMZ@GNPs and anti-EphA3-TMZ@GNPs were also subjected to NIR at an amount of 2 W/cm<sup>2</sup> for 5 minutes. Histological analysis of brain tissues indicated that tumours kept growing in both control (saline) and TMZ groups. The TMZ group exhibited a low apoptosis rate of around 11%, indicating that the tumour had developed resistance against TMZ. Contrarily, the groups subjected to NIR showed significantly higher rates of apoptosis around 82% and 88% for TMZ@GNPs and anti-EphA3-TMZ@GNPs, respectively. Collectively, these findings indicated that the activity of anti-EphA3-TMZ@GNPs group can perhaps be associated with anti-EphA3 as it increases the permeability and increases cellular uptake to the therapeutic agent [41].

## Brain-targeted aggregation-induced-emission nanoparticles with near-infrared imaging

Brain-targeted near-infrared IIb (NIR-IIb) aggregation-induced-emission (AIE) NPs have been developed for effective tracking of delivery systems. The brain-targeting peptide apolipoprotein E peptide (ApoE) is

grafted onto these NPs. After administration, these nanoparticles change to ApoE-Photothermal Nanoparticles (ApoE-Ph NPs) once subjected to NIR at 1550 nm wavelength. Adding ApoE to the NPs enhanced the cellular uptake of the ApoE-Ph NPs by 1.9 folds as compared to only Ph NPs. During the *in vitro* analysis using cell lines, ApoE-Ph NPs showed around 9.55% cell death, but irradiation with NIR increased the cell death up to 97.56%. Hence, the method seems to be more efficient, where cell death was observed to be around 88% at maximum. Moreover, ApoE-Ph NPs also exhibited a higher capability to bypass BBB [37].

Nanoparticles were administered in mice models through an injection in the tail vein. Bio-luminescence analysis aided by luciferase tagged cells in the brain, indicated that mice receiving ApoE-Ph NPs as well as NIR irradiation showed the highest tumour suppression. ApoE-Ph NPs showed a higher accumulation as compared to Ph NPs and the control (PBS). This indicates that ApoE-NPs are not only efficient in tumour suppression but may also need a smaller dose to achieve desired effects. Nanoparticles along with NIR offered a highly targeted and efficient solution for GB control and suppression [37].

## siRNA-based nanoparticles for targeting of oncogenic networks

Functionally active nanoparticles (NP-siRNA-CTX) were designed for effective delivery of the drugs. Nanoparticles consisted of an iron oxide core coated with a chitosan-PEG-PEI co-polymer that is conjugated to siRNA (short-interfering RNA) and a glioblastoma-targeting ligand chlorotoxin (CTX). Toxicity of nanoparticles was tested in C57BL/6J Wild-type mice by routine injections with NP-siMGMT-CTX. There were non-significant differences in the levels of biomarkers in rodent models injected with NP-siMGMT-CTX as well as in control mice. Collectively, these results suggested that treatment with NP-siMGMT-CTX neither impaired hematopoiesis nor disturbed renal and liver functions. These nanoparticles were found to be highly effective against orthotopic glioblastoma serially-passaged patient-derived xenograft rodent models. Importantly, these animal models have been demonstrated to display many of the histopathological properties that comprehensively characterize glioblastoma or experimentally recapitulate biological features of glioblastoma. Expression levels of MGMT in the tumour tissues of mice treated with NP-siMGMT-CTX were found to be reduced. Rodent models were intracranially transplanted with GBM6 cells. Later, the tumour-bearing rodent models were injected intravenously with NP-siMGMT-CTX. Furthermore, temozolomide was orally gavaged for enhanced combinatorial efficacy against glioblastoma.

Use of NP-siMGMT-CTX with temozolomide prolonged survival in a rodent model of glioblastoma serially-passaged patient-derived xenograft. Findings provided evidence of highly resistant and heterogeneous nature of GBM6 against temozolomide. More significantly, combinatorial treatments with siMGMT and temozolomide significantly enhanced the killing of glioblastoma and glioblastoma stem-like cells [38].

Ionisable cationic lipid nanoparticles (LNPs) with diverse amine headgroups have been shown to be promising candidates for brain-targeted siRNA delivery. BAMPA-O16B, a lipid has been shown to promote the cellular uptake and endosomal escape of siRNA lipoplexes in glioblastoma cells as well as in intracranial tumour tissues in a rodent orthotopic glioblastoma model. BAMPA-O16B/siRNA lipoplexes efficiently delivered siRNAs against CD47 and PDL1 across the blood-brain barrier into cranial GBM in mice, and repressed target gene networks in the tumour xenografts, resulting in synergistic activation of T cell-dependent anti-tumour immunity in orthotopic glioblastoma rodent models [20].

### Polymersomes as potent delivery systems for glioblastoma therapy

Discovery of immunogenic cell death (ICD) in the recent decades has revolutionized the field of molecular oncology and started to shed light on the significance of the signalling between dying cancer cells and the immune system in cancer therapy. Angiopep-2-conjugated pH-sensitive polymersomes (Au-DOX@PO-ANG) worked synergistically with X-rays and significantly induced ICD. Tumour vaccine-related experimental results clearly indicated that injections of dying necroptotic cancer cells effectively prevented tumour growth in tumour-bearing mice. Immunization of C57BL/6J rodent models with necroptotic G422 glioblastoma cells pretreated with Au-DOX@PO-ANG and radiation therapy were found to produce exciting results. Immunized C57BL/6J mice were tested for the efficacy of nanotechnologically delivered chemotherapeutic drugs. Immunization of the mice with G422 glioblastoma cells pretreated with Au-DOX@PO-ANG in combination with radiation therapy demonstrated notable signs of protection against tumour growth. More importantly, there was an evident reduction in tumour growth upon immunization of mice with G422 glioblastoma cells pretreated with Au-DOX@PO-ANG in combination with radiation therapy [13].

Loading of the chemotherapeutic drug (doxorubicin) in Apolipoprotein E (ApoE) peptide-functionalized reduction-sensitive polymersomes (ApoE-PS-DOX) potently inhibited tumour growths in orthotopic U87MG models. Animal models treated with ApoE20-PS-DOX demonstrated considerable DOX fluorescence throughout the tumour tissues. These animal model

studies emphasized the fact that ApoE20-PS-DOX proficiently promoted accumulation and retention of the drugs in intracranial glioblastoma tumours [26].

Pharmacologically efficient pH-sensitive polymersomes have been found to demonstrate significant tumour growth inhibitory effects in xenografted animal models. Initial studies had shown that under acidic environment, formation of the hydrogen bonds caused disruption of the core-shell structures, thus minimizing the stability of the polymersomes and release of the drugs. Secondly, gold nanoparticles and chemotherapeutic drug doxorubicin are nano-complexed as effective drug formulations. These types of complex drugs have two hallmark features. Gold nanoparticles are scientifically acclaimed radiosensitizers and effectively improve the radiotherapy effects in glioblastoma-related models and doxorubicin is widely used for the treatment of peripheral tumours. Hence, these complex drugs have synergistic and combinatorial functions and effects of radiotherapy and chemotherapy. Essentially, gold nanoparticles (NPs) and chemotherapeutic drug (doxorubicin) were encapsulated into the vesicles in stoichiometric ratios. Enhanced permeability and retention (EPR) effect is an exciting aspect associated while analysing the features of drug delivery systems. Polymersomes have been reported to demonstrate enhanced EPR effects. More importantly, the encapsulation within polymersomes overrides the challenging situation of quick clearance by EPR effects of tumour cells. Low-density lipoprotein receptor-related protein-1 (LRP1) is involved in pathogenesis. LRP1 is overexpressed on the endothelial cells of brain capillaries and glioblastoma cells. However, expression levels of LRP1 are low in the normal brain parenchymal cells. LRP1 has been shown to promote the entry of therapeutic drugs into central nervous systems through blood-brain barriers. Angiopep-2, a peptide mainly obtained from the Kunitz domains of aprotinin has unique functional properties to bind to LRP1. These features advocate the efficacy of Angiopep-2 as a potent molecule for LRP1-mediated targeted deliveries of the therapeutic drugs to glioblastoma. U87MG glioblastoma cells were transplanted into the right striatum for the construction of an orthotopically transplanted animal model. Au-DOX@PO-ANG nanoparticles significantly reduced the volume of tumour xenografts in tumour-bearing animal models. The side effects and toxicological effects of Au-DOX had been found to be minimized considerably after specific encapsulation in polymersomes. Therefore, targeted polymersomes caused reduction in the side effects and toxicological effects of Au-DOX. Findings obtained from staining revealed that DOX@PO-ANG-treated groups did not show pathological changes in heart, liver, spleen, lung and kidneys [14].

## Inhibition of oncogenic signalling pathways in glioblastoma

Synthetic protein nanoparticles (SPNPs) coated with the transcytotic peptide iRGD (AMD3100-SPNPs) for the targeting of CXCL2/CXCR4 cascade in glioblastoma cells have started to gain appreciation. It has been shown in a wide variety of cancers that frequent overexpression of CXCR4 on myeloid cells promoted trafficking of MDSCs (myeloid-derived suppressor cells). Enhanced infiltration of MDSCs not only promoted immunosuppressive tumour microenvironments but also contributed to the resistance against different immunotherapeutics. Therefore, blockade of CXCR4 provided a pharmacologically attractive target for an efficient immunotherapy. SPNPs loaded with AMD3100 (CXCR4 inhibitors) effectively blocked CXCR4-driven transduction cascade in aggressive intracranial GBM models. Systemically administered AMD3100-SPNPs caused a marked reduction in suppressive tumour immune microenvironment by suppression of the infiltration rates of inhibitory CXCR4<sup>+</sup> M-MDSCs [1].

## Conclusions

Several studies have been conducted in the recent years to assess the possibilities of using nanotechnology against GB. The approaches utilized by these studies are quite diverse. For example, some studies rely on polymer-based NPs due their better BBB crossing and cellular uptake capabilities, while others rely on metallic NPs that makes the use of imaging-based assessment procedures easier and make combinations of targeted therapies possible. However, most of the studies also utilized standard anti-cancer drugs like TMZ and DOX. The added ability of nanovectors makes the delivery of these drugs a lot more efficient, requiring even smaller doses and decreasing off-site effects. Furthermore, nanotechnology also helps tackle the drug resistance developed by different GB cases as specific proteins or genes can be suppressed by conjunction of an siRNA or a peptide with the anti-cancer drug containing NP. Similarly, nanotechnology has also made the highly targeted use of NIR and RT possible as NPs can specifically accumulate inside tumour cells, which can then be destroyed by NIR and RT. Hence, the side effects and off-site targeting of these procedures are also minimized. Moreover, in most of the cases NPs were administered via an injection in the tail vein of mice models. All the studies included in this article showed significant suppression of tumour volume during *in vivo* validations. Importantly, the use of NP-based approaches witnessed the least impact on the body weight of mice, and also witnessed an increase in the survival rate.

## Disclosure

The authors report no conflict of interest.

## References

- Alghamri MS, Banerjee K, Mujeeb AA, Mauser A, Taher A, Thalla R, McClellan BL, Varela ML, Stamatovic SM, Martinez-Revollar G, Andjelkovic AV, Gregory JV, Kadiyala P, Calinescu A, Jiménez JA, Apfelbaum AA, Lawlor ER, Carney S, Comba A, Faisal SM, Baris-si M, Edwards MB, Appelman H, Sun Y, Gan J, Ackermann R, Schwendeman A, Candolfi M, Olin MR, Lahann J, Lowenstein PR, Castro MG. Systemic delivery of an adjuvant CXCR4-CXCL12 signaling inhibitor encapsulated in synthetic protein nanoparticles for glioma immunotherapy. *ACS Nano* 2022; 16: 8729-8750.
- Aras A, Khokhar AR, Qureshi MZ, Silva MF, Sobczak-Kupiec A, Pineda EA, Hechenleitner AA, Farooqi AA. Targeting cancer with nano-bullets: curcumin, EGCG, resveratrol and quercetin on flying carpets. *Asian Pac J Cancer Prev* 2014; 15: 3865-3871.
- Attar R, Panah T, Romero MA, Yulaevna IM, Gazouli M, Berardi R, Wieczorek E, Farooqi AA. Overview of the signaling pathways involved in metastasis: An intriguing story-tale of the meta-static journey of ovarian cancer cells. *Cell Mol Biol (Noisy-le-grand)* 2021; 67: 212-223.
- Farhan M, Malik A, Ullah MF, Afaq S, Faisal M, Farooqi AA, Bier-sack B, Schobert R, Ahmad A. Garcinol sensitizes NSCLC cells to standard therapies by regulating EMT-modulating miRNAs. *Int J Mol Sci* 2019; 20: 800.
- Farooqi AA, Li Y, Sarkar FH. The biological complexity of RKIP signaling in human cancers. *Exp Mol Med* 2015; 47: e185.
- Farooqi AA, Nayyab S, Martinelli C, Berardi R, Katifelis H, Gazouli M, Cho WC. Regulation of Hippo, TGF- $\beta$ /SMAD, Wnt/ $\beta$ -catenin, JAK/STAT, and NOTCH by long non-coding RNAs in pancreatic cancer. *Front Oncol* 2021; 11: 657965.
- Farooqi AA, Qureshi MZ, Coskunpinar E, Naqvi SK, Yaylim I, Ismail M. MiR-421, miR-155 and miR-650: emerging trends of regulation of cancer and apoptosis. *Asian Pac J Cancer Prev* 2014; 15: 1909-1912.
- Gasparri ML, Attar R, Palaia I, Perniola G, Marchetti C, Di Dona-to V, Farooqi AA, Papadia A, Panici PB. Tumor infiltrating lymphocytes in ovarian cancer. *Asian Pac J Cancer Prev* 2015; 16: 3635-3638.
- Gasparri ML, Bardhi E, Ruscito I, Papadia A, Farooqi AA, Marchetti C, Bogani G, Ceccacci I, Mueller MD, Benedetti Panici P. PI3K/AKT/mTOR pathway in ovarian cancer treatment: are we on the right track? *Geburtshilfe Frauenheilkd* 2017; 77: 1095-1103.
- Gasparri ML, Besharat ZM, Farooqi AA, Khalid S, Taghavi K, Besharat RA, Sabato C, Papadia A, Panici PB, Mueller MD, Ferretti E. MiRNAs and their interplay with PI3K/AKT/mTOR pathway in ovarian cancer cells: a potential role in platinum resistance. *J Cancer Res Clin Oncol* 2018; 144: 2313-2318.
- Gooding M, Malhotra M, McCarthy DJ, Godinho BM, Cryan JF, Darcy R, O'Driscoll CM. Synthesis and characterization of rabies virus glycoprotein-tagged amphiphilic cyclodextrins for siRNA delivery in human glioblastoma cells: *in vitro* analysis. *Eur J Pharm Sci* 2015; 71: 80-92.
- Han M, Xing H, Chen L, Cui M, Zhang Y, Qi L, Jin M, Yang Y, Gao C, Gao Z, Xing X, Huang W. Efficient antiglioblastoma therapy in mice through doxorubicin-loaded nanomicelles modified using a novel brain-targeted RVG-15 peptide. *J Drug Target* 2021; 29: 1016-1028.
- He C, Ding H, Chen J, Ding Y, Yang R, Hu C, An Y, Liu D, Liu P, Tang Q, Zhang Z. Immunogenic cell death induced by chemoradiotherapy of novel pH-Sensitive cargo-loaded polymersomes in glioblastoma. *Int J Nanomedicine* 2021; 16: 7123-7135.
- He C, Zhang Z, Ding Y, Xue K, Wang X, Yang R, An Y, Liu D, Hu C, Tang Q. LRP1-mediated pH-sensitive polymersomes facilitate

- combination therapy of glioblastoma in vitro and in vivo. *J Nanobiotechnology* 2021; 19: 29.
15. He W, Li X, Morsch M, Ismail M, Liu Y, Rehman FU, Zhang D, Wang Y, Zheng M, Chung R, Zou Y, Shi B. Brain-targeted codelivery of Bcl-2/Bcl-xl and Mcl-1 inhibitors by biomimetic nanoparticles for orthotopic glioblastoma therapy. *ACS Nano* 2022; 16: 6293-6308.
  16. Katifelis H, Nikou MP, Mukha I, Vityuk N, Lagopati N, Piperi C, Farooqi AA, Pippa N, Efsthopoulos EP, Gazouli M. Ag/Au bimetallic nanoparticles trigger different cell death pathways and affect damage associated molecular pattern release in human cell lines. *Cancers (Basel)* 2022; 14: 1546.
  17. Li X, Xu H, Li C, Qiao G, Farooqi AA, Gedanken A, Liu X, Lin X. Zinc-doped copper oxide nanocomposites inhibit the growth of pancreatic cancer by inducing autophagy through AMPK/mTOR pathway. *Front Pharmacol* 2019; 10: 319.
  18. Lin X, Khalid S, Qureshi MZ, Attar R, Yaylim I, Ucak I, Yaqub A, Fayyaz S, Farooqi AA, Ismail M. VEGF mediated signaling in oral cancer. *Cell Mol Biol (Noisy-le-grand)* 2016; 62: 64-68.
  19. Liu PF, Farooqi AA, Peng SY, Yu TJ, Dahms HU, Lee CH, Tang JY, Wang SC, Shu CW, Chang HW. Regulatory effects of noncoding RNAs on the interplay of oxidative stress and autophagy in cancer malignancy and therapy. *Semin Cancer Biol* 2022; 83: 269-282.
  20. Liu S, Liu J, Li H, Mao K, Wang H, Meng X, Wang J, Wu C, Chen H, Wang X, Cong X, Hou Y, Wang Y, Wang M, Yang YG, Sun T. An optimized ionizable cationic lipid for brain tumor-targeted siRNA delivery and glioblastoma immunotherapy. *Biomaterials* 2022; 287: 121645.
  21. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* 2007; 114: 97-109. Erratum in: *Acta Neuropathol* 2007; 114: 547.
  22. Mao X, Calero-Pérez P, Montpeyó D, Bruna J, Yuste VJ, Candiota AP, Lorenzo J, Novio F, Ruiz-Molina D. Intranasal administration of catechol-based Pt(IV) coordination polymer nanoparticles for glioblastoma therapy. *Nanomaterials (Basel)* 2022; 12: 1221.
  23. Naoum GE, Buchsbaum DJ, Tawadros F, Farooqi A, Arafat WO. Journey of TRAIL from bench to bedside and its potential role in immuno-oncology. *Oncol Rev* 2017; 11: 332.
  24. Nogueira DR, Scheeren LE, Macedo LB, Marcolino AI, Pilar Vinar-dell M, Mitjans M, Rosa Infante M, Farooqi AA, Rolim CM. Inclusion of a pH-responsive amino acid-based amphiphile in methotrexate-loaded chitosan nanoparticles as a delivery strategy in cancer therapy. *Amino Acids* 2016; 48: 157-168.
  25. Ou A, Ott M, Fang D, Heimberger AB. The role and therapeutic targeting of JAK/STAT signaling in glioblastoma. *Cancers (Basel)* 2021; 13: 437.
  26. Ouyang J, Jiang Y, Deng C, Zhong Z, Lan Q. Doxorubicin delivered via ApoE-directed reduction-sensitive polymersomes potently inhibit orthotopic human glioblastoma xenografts in nude mice. *Int J Nanomedicine* 2021; 16: 4105-4115.
  27. Prados MD, Berger MS, Wilson CB. Primary central nervous system tumors: advances in knowledge and treatment. *CA Cancer J Clin* 1998; 48: 331-360, 321.
  28. Qiao G, Xu H, Li C, Li X, Farooqi AA, Zhao Y, Liu X, Liu M, Stagos D, Lin X. Granulin synergizes with cisplatin to inhibit the growth of human hepatocellular carcinoma. *Int J Mol Sci* 2018; 19: 3060.
  29. Reifenberger G, Reifenberger J, Ichimura K, Meltzer PS, Collins VP. Amplification of multiple genes from chromosomal region 12q13-14 in human malignant gliomas: preliminary mapping of the amplicons shows preferential involvement of CDK4, SAS, and MDM2. *Cancer Res* 1994; 54: 4299-4303.
  30. Renziehausen A, Tsailanis AD, Perryman R, Stylos EK, Chatzi-giannis C, O'Neill K, Crook T, Tzakos AG, Syed N. Encapsulation of temozolomide in a calixarene nanocapsule improves its stability and enhances its therapeutic efficacy against glioblastoma. *Mol Cancer Ther* 2019; 18: 1497-1505.
  31. Saleem M, Abbas K, Manan M, Ijaz H, Ahmed B, Ali M, Hanif M, Farooqi AA, Qadir MI. Review-Epigenetic therapy for cancer. *Pak J Pharm Sci* 2015; 28: 1023-1032.
  32. Smith JS, Alderete B, Minn Y, Borell TJ, Perry A, Mohapatra G, Hosek SM, Kimmel D, O'Fallon J, Yates A, Feuerstein BG, Burger PC, Scheithauer BW, Jenkins RB. Localization of common deletion regions on 1p and 19q in human gliomas and their association with histological subtype. *Oncogene* 1999; 18: 4144-4152.
  33. Sousa F, Dhaliwal HK, Gattacceca F, Sarmento B, Amiji MM. Enhanced anti-angiogenic effects of bevacizumab in glioblastoma treatment upon intranasal administration in polymeric nanoparticles. *J Control Release* 2019; 309: 37-47.
  34. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005; 352: 987-996.
  35. Tan AC, Ashley DM, López GY, Malinzak M, Friedman HS, Khasraw M. Management of glioblastoma: State of the art and future directions. *CA Cancer J Clin* 2020; 70: 299-312.
  36. Tanriover G, Dilmac S, Aytac G, Farooqi AA, Sindel M. Effects of melatonin and doxorubicin on primary tumor and metastasis in breast cancer model. *Anticancer Agents Med Chem* 2022; 22: 1970-1983.
  37. Wang J, Liu Y, Morsch M, Lu Y, Shangguan P, Han L, Wang Z, Chen X, Song C, Liu S, Shi B, Tang BZ. Brain-targeted aggregation-induced-emission nanoparticles with near-infrared imaging at 1550 nm boosts orthotopic glioblastoma theranostics. *Adv Mater* 2022; 34: e2106082.
  38. Wang K, Kievit FM, Chiarelli PA, Stephen ZR, Lin G, Silber JR, Ellenbogen RG, Zhang M. siRNA nanoparticle suppresses drug-resistant gene and prolongs survival in an orthotopic glioblastoma xenograft mouse model. *Adv Funct Mater* 2021; 31: 2007166.
  39. Yen CY, Huang HW, Shu CW, Hou MF, Yuan SS, Wang HR, Chang YT, Farooqi AA, Tang JY, Chang HW. DNA methylation, histone acetylation and methylation of epigenetic modifications as a therapeutic approach for cancers. *Cancer Lett* 2016; 373: 185-192.
  40. Yenilmez EN, Genc D, Farooqi AA, Tunoglu S, Zeybek U, Akkoc T, Yaylim I. Mesenchymal stem cells combined with IFN induce apoptosis of breast cancer cells partially through TRAIL. *Anticancer Res* 2020; 40: 5641-5647.
  41. Yu Y, Wang A, Wang S, Sun Y, Chu L, Zhou L, Yang X, Liu X, Sha C, Sun K, Xu L. Efficacy of temozolomide-conjugated gold nanoparticle photothermal therapy of drug-resistant glioblastoma and its mechanism study. *Mol Pharm* 2022; 19: 1219-1229.