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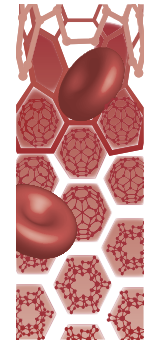

An update of nanoparticle-based approaches for glioblastoma multiforme immunotherapy

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Glioblastoma multiforme is a serious medical issue in the brain oncology field due to its aggressiveness and recurrence. Immunotherapy has emerged as a valid approach to counteract the growth and metastasization of glioblastoma multiforme. Among the different innovative approaches investigated, nanoparticles gain attention because of their versatility which is key in allowing precise targeting of brain tumors and increasing targeted drug delivery to the brain, thus minimizing adverse effects. This article reviews the progress made in this field over the past 2 years, focusing on nonspherical and biomimetic particles and on vectors for the delivery of nucleic acids. However, challenges still need to be addressed, considering the improvement of the particles passage across the blood–meningeal barrier and/or the blood–brain barrier, promoting the clinical translatability of these approaches.

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Introduction to the clinical issue

Glioblastoma multiforme (GBM) is the most common malignant tumor of the CNS, representing about 65% of all primary CNS malignancies [1] and 82% of cases of malignant glioma [2]. Despite this, the incidence (3.1/100,000 per year) is low in comparison with other non-neural cancers and it is more frequent in people older than 75 years [3]. The 2007 WHO classification of tumors of CNS split gliomas into four grades of aggressiveness, and GBM and its variants were classified as grade IV tumors [4]. GBMs can either start from normal brain cells or develop from an existing low-grade astrocytoma. Indeed, GBM is also defined as a grade IV astrocytoma.

From a molecular point of view, malignant gliomas are highly heterogeneous tumors [5]. They may be divided into four molecular subclasses (classical, mesenchymal, proneural and neural) based on the transcriptional pathways and the mutations of several genes, including isocitrate dehydrogenase, EGFR, tumor protein 53 and NF-κB [6].

Within the definition of GBM, it is possible to distinguish primary and secondary GBMs, which develop at different ages, carry specific molecular alterations and differ in terms of histology, localization, grade of necrosis and metastasization, responsiveness to therapies and clinical outcome. This is the reason why many studies suggest that primary and secondary GBM should be considered as different tumor entities [7].

GBMs is usually diagnosed using CT scan, MRI scan or tissue biopsy, or a combination of the three. The current standard of care involves surgery followed by radiotherapy, with concomitant cycles of temozolomide chemotherapy [8]. High-dose steroids may also reduce symptoms, like brain swelling, but GBM usually recurs after some time. Without treatment, the median survival is about 3 months from diagnosis, while treated patients' life expectancy is 12–15 months. Unfortunately, less than 3–7% of treated patients survive over 5 years following diagnosis [9]. The tumor recurrence is attributable to heterogeneity, therapy resistance, high angiogenesis and the high invasiveness of GBM stem cells.

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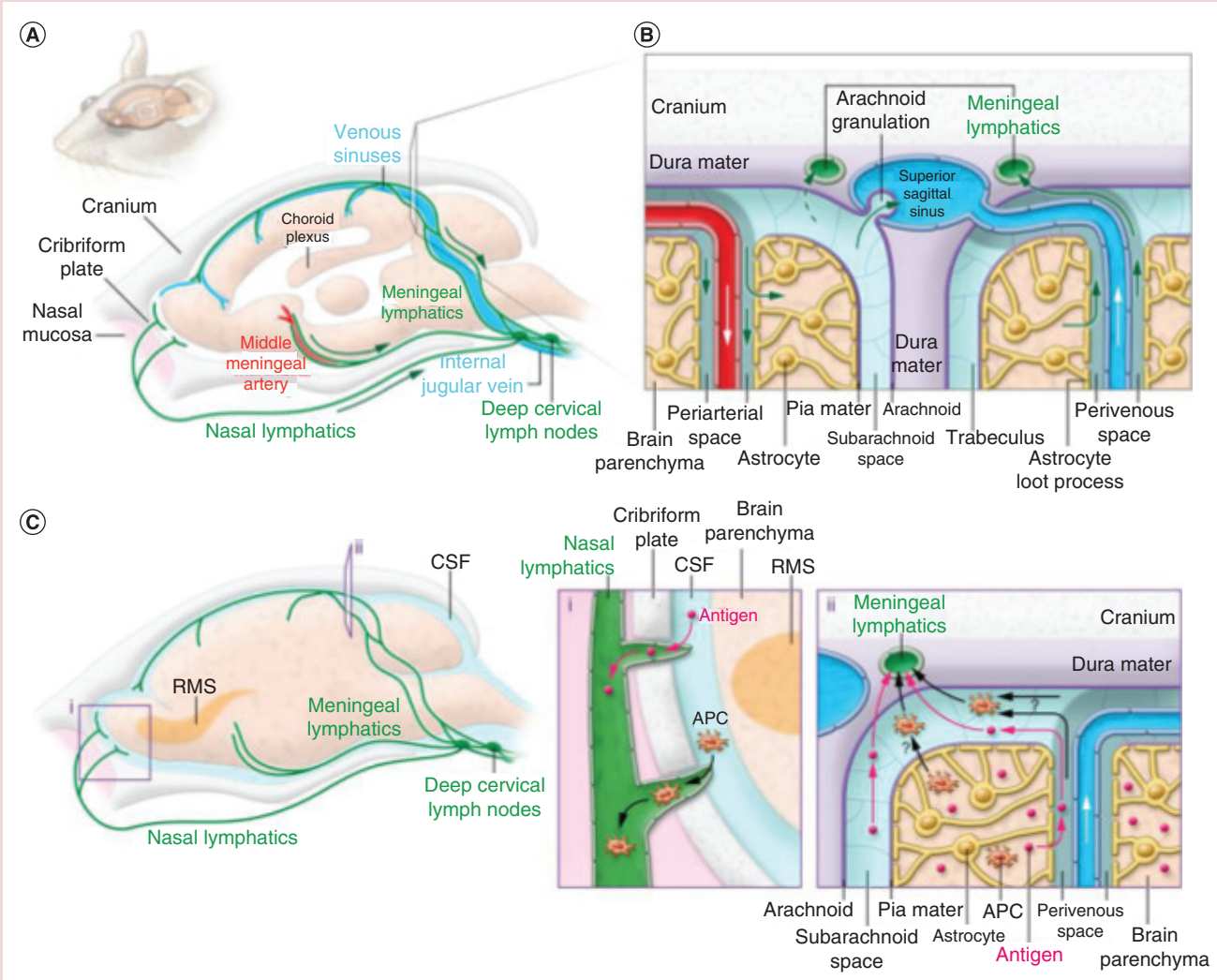

Figure 1. Illustration of the presumptive network of vessels in cerebral glymphatic system and lymphatic drainage, together with cerebrospinal fluid and interstitial fluid circulations across the brain.
APC: Antigen-presenting cells; CSF: Cerebrospinal fluid; RMS: Rostral migratory stream.
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The immune system in the brain

The CNS has been traditionally considered an immune privileged system because it lacks a classical lymphatic system and because of the difficulty in starting a destructive T-cell response from parenchyma [10]. However, recent findings have shown that the presence of a functional meningeal system (located in the dura mater) allows a flow of molecules and immune cells into the deep cervical lymph nodes [11]. The structures that express all the molecular hallmarks of lymphatic endothelial cells were deeply analyzed by Iliff *et al.* in 2012 [12], and by Louveau *et al.* in 2015 [13], and now they are commonly known as the 'glymphatic system'. The exact extent of the network (Figure 1, reproduced from Louveau *et al.*) is still unknown [14] but further analysis revealed a complex system of perivascular tunnels, basement membranes [15] and astroglial cells that allow the continuous exchange of cerebrospinal fluid and interstitial fluid along the periarterial space, including macromolecules and solutes [16]. Moreover, other studies showed a specific paravascular compartment for small lipid transport and glial communication signaling [17].

Originally, CNS immune privilege was partly attributed to the lack of a classical lymphatic system, although allografts that established an immune response in peripheral organs were able to maintain and potentiate that

response when implanted into brain parenchyma [19]. Brain parenchyma and meningeal compartment are very different in terms of properties, starting from the fact that the blood–meningeal barrier is more permissive than the blood–brain barrier (BBB), which is why immune cells are free to circulate only within meningeal spaces under physiological conditions [18]. Despite that, Shechter *et al.* proposed a model in which selective barriers such as the BBB or blood–testis barrier do not represent static structures only able to segregate immune cells outside the organs but, rather, they are permissive gates that regulate the passage of cells under specific conditions, for example in some particular phenotypes. In this context, the privilege of CNS is not the power of exclusion, but the ability to build an effective communication with the active immune system [20].

In fact, recent studies demonstrate that antigens can drain from the brain to deep cervical lymph nodes, via meningeal lymphatic vasculature, throughout an internal recirculation mechanisms involving cerebrospinal fluid and interstitial fluid in order to initiate the immune response [14]. However, since the response is slow, there is a need for a large amount of antigen or a secondary signaling to trigger this response. Alternatively the cervical lymph nodes may have the property to modulate the immune response to CNS antigens either toward tolerance or reactivity [18].

GBM immunotherapy

The tumor microenvironment (TME) is a complex network of extracellular matrix factors and cells, in which tumor cells can resist, proliferate and invade healthy tissues. The immune response is a crucial factor for communication between GBM and TME and tumor progression.

It has been reported that nascent tumor cells can be eliminated by the host immune system based on both innate and adaptive immunity, opening the possibility to approach GBM by immunotherapies [21]. Immunotherapy has gained a lot of consideration in the last years and has become one of the most valid choices for cancer treatment, since it represents a better approach to prevent metastasization and recurrence in respect to conventional drugs. Immunotherapy includes the use of vaccines, oncolytic viral therapies, immune checkpoint inhibitors (ICIs) and chimeric antigen receptor T-cell (CAR-T) therapy [22].

Immune checkpoints, such as cytotoxic T lymphocyte-associated protein 4, protein death 1 (PD-1) and protein death ligand 1 (PD-L1) are negative modulators of T-cell activation which is why they have recently been the targets of drugs in clinical practice [10]. In GBM, cancer cells are able to escape immune surveillance through changes in receptor expression. For this reason, ICIs have become an intriguing subject to explore. PD-L1 is present in a variable subset of GBMs (between 2 and 88%) and its higher expression has shown to correlate with a poor prognosis [23].

Some studies have demonstrated that tumor-infiltrating $CD8^+$ T lymphocytes and intratumoral TH1-type molecules are associated with positive therapeutic outcome by blockading PD-1 and PD-L1. However, therapies with an antibody targeting PD-1 (anti-PD-1) displayed response rates from 17 to 21%, probably due to the tumor heterogeneity and to the fact that immune responses and GBM are not caused by a single immune cell or checkpoint, but by multiple more complex interactions [24]. Indeed, cocktails of ICIs have demonstrated, in preclinical or clinical studies, a huge activity alone or in combination with traditional therapies, increasing overall survival and providing a good safety profile [22]. This opens the possibility to further guide the individualized treatment of patients by generating personalized medicines.

Innovative approaches: nanoparticles

Existing cancer immunotherapies have limited clinical benefits because of side effects (e.g., autoimmune diseases) and because cancer antigens are often not effectively delivered to immune cells. In particular, for solid tumors, like GBM, immunotherapy is less effective than in lymphoma due to the difficulty of immune cell penetration within the abnormally grown extracellular matrix. In addition, the TME is a hostile environment for immune response because of the presence immune-suppressive factors (e.g., tumor-secreted cytokines). These limitations can be overcome by using nanoparticles (NPs) for cancer immunotherapy.

NPs are small structures, with a size range between 1 and 100 nm, made by either inorganic, polymeric or organic materials which can be loaded with drugs [25]. Their physical and chemical properties make them attractive for many uses, especially in the medical field [26], where they have been successfully applied to treat several diseases, cancer included [27]. An overview of NPs exploited for drug delivery in cancer therapy is shown in Figure 2 (reproduced from Sun *et al.*) [25].

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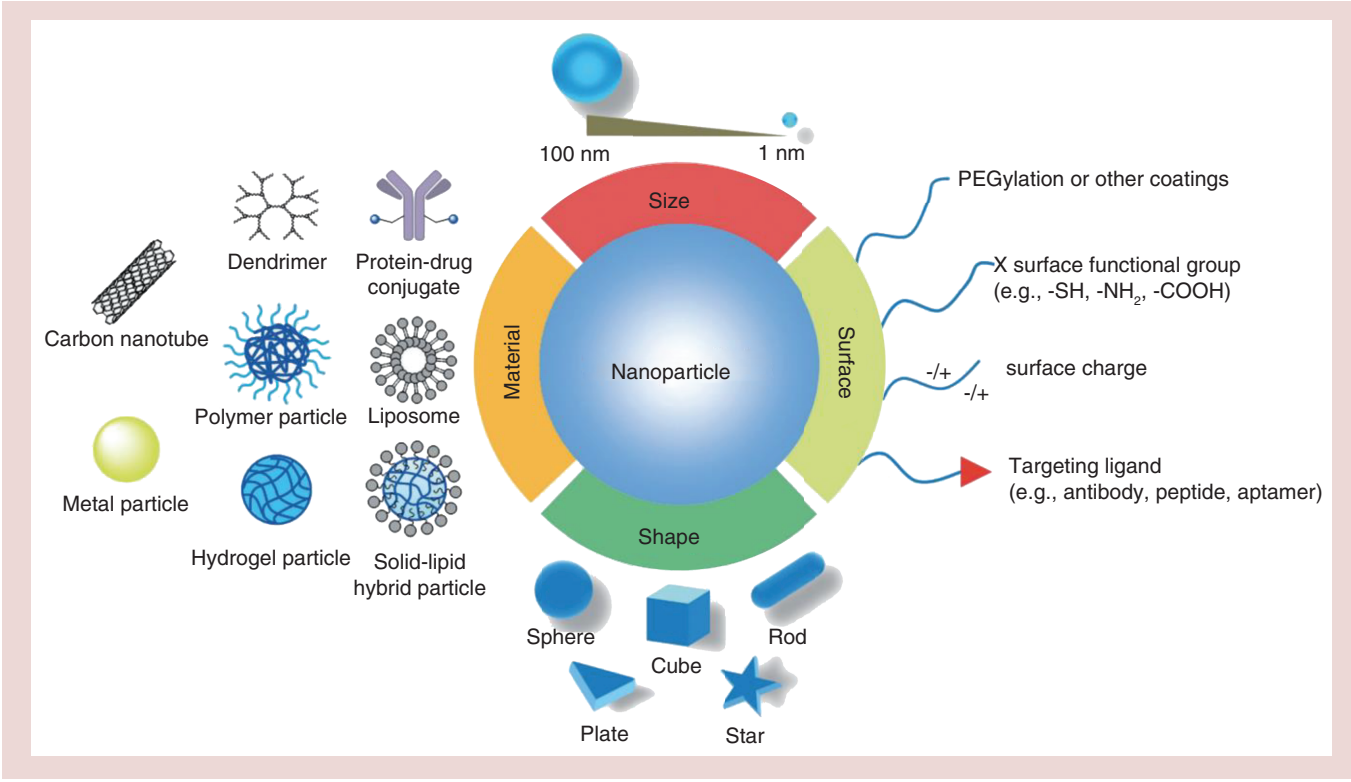

Figure 2. General overview of the main types of nanoparticles applied in cancer therapy and their possible functionalizations.
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To achieve effective immunotherapy, tumor antigens need to reach the lymph nodes in an efficient way, so that the immune response against cancer can start. Thus, NPs represent the best-characterized delivery vehicle to make sure tumor antigens reach lymph nodes. A specially functionalized NP can induce, inhibit or alter the innate immune system, for example by inducing cytokine production, activating downregulation mechanisms or immunosuppressing immune cells [28–31].

Beyond tumor antigens, NPs can efficiently deliver adjuvants to antigen-presenting cells situated in the lymph nodes, allowing antigen presentation. Accordingly, NP-based immunotherapy can provide a long-lasting vaccine effect and a wide range of immune responses as well, contrarily to conventional immunotherapy.

However, because of the heterogeneity and complexity of brain diseases, GBM included, each NP has to be efficiently designed both to overcome the BBB (even if it is disrupted in some disease conditions and brain areas) and to specifically target cancer cells. However, these issues are difficult to be achieved due to the absence or low density of BBB-ligands and cancer-specific receptors. In fact, the choice of the tumor-specific ligand to be targeted by NPs strongly depends on its level of expression, rate of recycling, cellular localization and then its accessibility. All these factors are important in NP design because they determine the efficacy and safety of NPs themselves [32]. The challenge for the future will be to associate the diagnosis with the therapy in order to identify the targeted ligand that is overexpress in diseases tissue and then design the NP early [33–35].

2018-2020: what's new?

Nonspherical NPs & biomimetics for drug delivery

Although liposomes (spherical NPs) are one of the most used NPs in nanomedicine, nonspherical NPs (like discoidal particles, nanorods and filamentous particle) seem to display better performances in tumor treatment in terms of their ability to avoid the uptake by macrophages in organs and vessels, cellular uptake and biodistribution and their ability to cross biological barriers, the BBB included [36,37].

In one study, verteporfin (VP), a benzoporphin-derived small molecule, was encapsulated in a micellar vehicle composed in poly(ethylene glycol)-poly(-amino ester)-poly(ethylene glycol) (PEG-PBAE-PEG) in order to improve

stealthiness and to avoid protein corona formation [38]. VP was chosen since it has been shown that this molecule can interfere with GBM cell growth and proliferation [39]. Contrarily to spherical micelles containing VP, drug delivery mediated by filamentous micelles containing VP (fVPs) was proven to induce specific cytotoxicity on GBM cells, saving normal human astrocytes. Moreover, it has been shown by the same research group [40], that fVPs better avoid macrophage uptake *in vitro*, thus prolonging their circulation in the blood. Interestingly, a single treatment with fVPs in an ectopic xenograft tumor model has shown more than double the accumulation within the tumor in respect to the treatment with spherical micelles containing VP, demonstrating that nonspherical NPs can be a better tool for drug delivery [38].

The cellular uptake and targeting properties of NPs are essential to maximize their specificity toward target tissues. In comparison with spherical NPs, nonspherical ones display a stronger targeting avidity due to their ability to form a higher number of multivalent interactions between the ligand on the NP's surface and the target molecule on the tumor cell surface [41], GBM included [42].

Moreover, thanks to the intrinsic properties of nonspherical NPs (high aspect ratio and prolonged lifetime in the blood circulation), the extravasation rate, the penetration capacity and the margination effect within solid tumors are improved when compared with those of spherical ones.

All these features, together with size, can make nonspherical NPs better in terms of crossing the BBB, as suggested by Dal Magro *et al.* who demonstrated that discoidal NPs can be more efficiently transcytosed across the BBB *in vitro*, in respect to their spherical counterparts [37]. This issue is of a particular relevance for the GBM treatment.

Taken together, these peculiarities allow more efficient drug delivery and probably for this reason, the immune response results are increased, even if this is an indirect effect of NPs shape.

Another strategy to improve the tumor-targeting ability is the design of biomimetic NPs, which are small structures that imitate the characteristics of biological entities physiologically present in human body [43]. They are widely used as a valid tool for cancer nanomedicine.

HDLs are heterogeneous particles naturally in the form of nanodiscs, involved in reverse cholesterol transport [44]. They may be used for drug-delivery purposes, providing accumulation and diffusion through the tumor because of their small size. In a study of 2019, therapeutic drugs were used to elicit tumor cell death and antiglioma immunity [45]. In this study, Kadiyala *et al.* set up HDL-mimicking nanodiscs conjugated to CpG (a TLR9 ligand expressed by several immune cells able to trigger immune rejection) and loaded with docetaxel (DTX), a chemotherapeutic agent [45]. DTX-sHDL-CpG nanodiscs were demonstrated to trigger antitumor $CD8^+$ T cell-mediated immunity and to develop a long-term immunological memory; in fact, treated mice remained tumor-free when the contralateral hemisphere was injected a second time with the tumor cells.

Among biomimetic NPs, extracellular vesicles (EVs), originating from the endosomal system are considered good candidates for intercellular communication and exchange [46], since they contain proteins from their cell type of origin. Zhu *et al.* have evaluated the antitumor activity and the tumor-targeting ability of EVs derived from natural killer (NK) cells pre-exposed to IL-15, so called NK-$EVs_{IL\text{-}15}$, in comparison with EVs isolated from naive NK cells (NK-EVs) [47]. It is known that IL-15 is able to improve survival and activation of NK cells [48]. NK-$EVs_{IL\text{-}15}$ were demonstrated to express more cytotoxic proteins such as perforin and FasL than NK-EVs. Further, the accumulation of NK-EVsIL-15 in the GBM area *in vivo* was double that of NK-EVs. Moreover, the antitumor effect was improved, but it was not stable after the interruption of the treatment.

The same group explored the antitumor activity of exosome-mimetic (EMs) vesicles derived from NK cells (NK-EMs) *in vitro* and *in vivo* in a xenograft mouse tumor model of GBM [49]. NK-EMs were prepared by disrupting NK cells through serial extrusions using nanosized filters. NK-EMs combine the characteristics of cells and exosomes and their cytotoxicity in various cancer cell lines, including GBM, was shown to be superior. On the contrary, exosomes directly derived from NK cells (NK-Exo) come from NK cells culture medium and they do not show the same characteristics. Furthermore, EMs were shown to efficiently cross the BBB and to provide tumor targetability and cytotoxicity in a GBM xenograft model.

Another biomimetic delivery system is an albumin-based structure for the co-delivery of disulfiram (a copper chelating agent) and the macrophage modulator regorafenib. This structure has been used to simultaneously target glioma cells and protumor M2 macrophages [50]. In this case, albumin can target SPARC proteins, which are overexpressed on tumor cells and on tumor-associated blood vessels (i.e., on endothelial cells). Moreover, in the study, transferrin receptor-binding peptide T12 was used to enhance traversal of the BBB and uptake into glioma cells. This approach was demonstrated to induce cytotoxic T lymphocyte immune responses and the suppression of

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Table 1. Different types of nanoparticles that may be used to deliver nucleic acid for cancer therapy.

Type of Nanoparticle	Advantages	Disadvantages	Ref.
Lipid-based	Allow efficient condensation and encapsulation of negatively charged molecules such as nucleic acids	Cannot actively reach the tumor or the tumor microenvironment without functionalization	[56]
Natural, semi-synthetic or synthetic polymer-based	Can bind nucleic acids through electrostatic forces and are efficient in gene delivery	Polymers can be easily inactivated by opsonization or fibrinogen in the circulatory system. To avoid this, polyethylene glycol, RGD peptide or other functional groups must be added	[54,56]
Gold	Easy to prepare and stable. They can be efficiently functionalized and conjugated	May show high cytotoxicity	[54]
Cyclodextrin-based	Water soluble and capable of forming complexes with nucleic acids	Have low stability and a tendency to aggregate and fall apart upon dilution	[55]
Chitosan	Show low immunogenicity and high biodegradability and biocompatibility	Have low solubility at physiological pH and cannot easily release drugs in a controlled manner	[55,56]

RGD: Arginylglycylaspartic acid peptide.

protumor M2 macrophages. Notably, a combination of chemotherapy and immunotherapy has shown to improve the treatment outcomes [51].

NPs for the delivery of nucleic acids

Gene therapy involves the use of nucleic acids (DNA or RNA) to treat, cure or prevent disorders using different tools such as naked oligonucleotides, viral and nonviral vectors [52]. Although the idea is simple, in theory, it is actually subject to multiple factors which make it very complex. For brain diseases, the most commonly used viral vectors are adeno-associated viruses and lentiviruses, while the most commonly used nonviral vectors are naked plasmid DNA and complexes with polymers or cationic lipids [53]. An overview of some nonviral NPs for the delivery of nucleic acids is shown in Table 1 [54–56].

In a recent study, *in vitro*-transcribed mRNA was formulated into an injectable therapeutic with the purpose to reprogram tumor-associated macrophages (TAMs) [57]. In fact, M1 macrophages are known as antitumoral cells, while M2 macrophages act as protumoral cells [58]. *In vitro*-transcribed mRNAs can efficiently target genes useful to shift the macrophages phenotype from M2-like to M1-like, thus promoting their genetic reconfiguration. These nucleic acids (two mRNAs encoding interferon regulatory factor 5 and an interferon regulatory factor 5 kinase were encapsulated into biodegradable polymeric PBAE NPs providing a reduction of tumor progression in glioma mouse models.

In 2018 and 2019, Kim *et al.* improved the efficacy of ICIs in GBM by restoring p53 functions through the delivery of *TP53* gene via a novel encapsulating plasmid cationic liposome (SGT-53) [59,60]. These nonviral vectors were functionalized with a single chain antibody fragment recognizing transferrin receptor that is more expressed by cancer cells than healthy ones, thus enhancing the targeting performance of the NPs. The treatment was performed in co-administration with anti-PD-1 antibody both *in vitro* and *in vivo* in syngeneic mouse models of GBM. Restoration of p53 function improved the anti-PD-1 response, modified the TME by increasing lymphocytes tumor-infiltration and shifted the macrophage phenotype from M2 to M1.

The reason why many patients develop a resistance to ICIs is because GBM is basically an immunologically 'cold' tumor, meaning there is a limited presence of tumor-infiltrating lymphocytes. However, as Baratta noticed, this kind of tumors can be successfully infiltrated by antigen-specific T cells that have the potential to kill cancer cells and to turn the 'cold' tumor into a 'hot' one, for example by using personalized vaccines and combined treatments [61]. This idea has been pursued through the use of plant virus-like particles derived from Cowpea mosaic virus to promote an *in situ* vaccine immunotherapy against malignant glioma by triggering antitumor responses [62]. In this study, brain injection of Cowpea mosaic virus in syngeneic glioma mouse models provided a significant increase in infiltration of $CD8^+$ T cells, effector memory $CD8^+$ T cells and NKT cells (unique innate T cells that express markers for T and NK cells) [63].

Beyond mRNAs, other nucleic acids can be delivered into tumor cells. A siRNA is a short double-stranded RNA from 21 to 23 nucleotides length, which contains a sequence of mRNA (sense strand) and its complement (antisense active strand) [64]. siRNAs can inhibit complementary post-transcriptional mRNAs by forming RNA-induced silencing complexes. Together with the RNase III enzyme dicer, these can promote endonucleolytic cleavage of target mRNA strands [65].

It has been shown that the incorporation of disulfide bonds in PBAE polymer NPs is able to ameliorate siRNA delivery in patient-derived GBM cells in co-culture with brain capillary endothelial cells. This modification promotes triggered release of siRNA into the intracellular space and has reduced risk of cytotoxicity. In a mouse model of GBM, these NPs have been proven to be safe, although further investigation about efficacy is needed [66].

In a study of 2019, five siRNAs were included in a single type of PBAE-based NP (R646 siRNA NPs) to knockdown different genes known to promote proliferation and migration (YAP1, NKCC1, survivin, Robo1 and EGFR) in GBM cells [67]. These siRNAs were included in the PBAE-based NPs at a very low dose. For this reason, the authors could not test the efficacy, but instead they focused their attention on safety and specificity for GBM cells over healthy cells. Thus, although further investigations are needed, this kind of delivery system seems to be promising. Moreover, in tumor mouse models a reduction of tumor burden over the time was seen after siRNA-NP administration.

At last, an alternative nonviral gene delivery agent is represented by solid lipid NPs (SLNs). These are colloidal nanocarriers made up of a high melting fat matrix and a monolayer of phospholipids acting as a surfactant [68]. iRGD, a cyclic peptide with high affinity to vascular endothelial cells α_v integrins, can be conjugated to SLNs embedding siRNAs against EGFR and PD-L1 in *in vitro* and *in vivo* models of GBM [69]. Furthermore, it was shown that a short burst of radiation therapy was able to improve the tumor uptake of SLNs. This delivery system provided activation of the immune response, inhibition of tumor growth and an increase in mouse survival.

Other NP-based strategies

Possible strategies to design different NPs are virtually boundless. Sometimes NP design is aimed to compensate a lack of response from free drugs, for example when they are not able to diffuse inside the target cells because they are polar molecules. On the other hand, the design of a particular NP may be made to provide a better response from drugs. For example, many patients with solid cancers do not respond to CAR-T cells therapies [70]. This is because solid cancers are able to suppress T-cell functions by secreting inhibitory factors into their TME [71]. Many studies have tried to remedy this issue: one strategy was presented by Zhang *et al.* in 2018 by designing liposomes containing a drug cocktail of PI-3065/7DW8-5 (a PI3K inhibitor and an indirect immunostimulator of natural killer T [NKT] cells, respectively) followed by engineered CAR-T cells infusion. This can block suppressor cells within the TME and at the same time can stimulate key antitumor immune cells [72]. These NPs were proven to be effective *in vitro* and *in vivo* in a mouse model of glioma, transiently resetting TME, and they might be a valid approach for clinical trials.

Immunosuppression of GBM does not involve only T cells, but also TAMs and myeloid-derived suppressor cells. This is often due to PD-1/PD-L1 pathway, which promotes, for example, the immunosuppressive mechanism of TAMs to counteract the antitumor activity of T cells [73]. Since PD-L1 is often overexpressed on GBM cells, Zhang *et al.* proposed the functionalization of a lipid NP with anti-PD-L1 antibodies in order to effectively deliver dinaciclib (a CDK5 inhibitor) to tumor-associated myeloid cells (TAMCs, which include myeloid-derived suppressor cells and TAMs) [74]. Combined treatment of mice with radiotherapy that elicits upregulation of PD-L1, enhanced the targeting efficiency of these lipid NPs and provided a better delivery of dinaciclib to TAMCs. This approach led to increased cytotoxicity and depletion of immunosuppressive TAMCs, benefitting tumor-bearing mice.

At last, a device formed by nanodiamonds with surface functionalization of polyglycerol loaded with doxorubicin (Nano-DOX) has been developed to reprogram immunosuppressive TME of GBM and to induce anticancer immune response [75]. Activation of autophagy, instead of apoptosis, was confirmed in Nano-DOX-treated GBM cells and in xenograft models. Moreover, dendritic cells-stimulated T-cell activation was shown both *in vitro* and *in vivo*.

Conclusions & future perspective

In the last 2 years, many strategies to improve targeted drug delivery via NPs have been proposed. However, further studies and insights are needed in the most part of published papers, some proposals are very promising, especially from the clinical translatability point of view.

Biomimetics, for example, seems to be a valid tool because of their intrinsic characteristics, including replicating already existing cellular structures, thus increasing their safeness. As a matter of fact, the side effects of these treatments are remarkably reduced and there is in theory the possibility to create countless variants of these NPs.

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Likewise, nonspherical NPs provide better performance in cell interactions and in crossing biological barriers. Consequently, this finding can provide better strategies to pursue in order to improve drug delivery.

Combined treatments and NPs functionalization can serve multiple needs for different cellular types and even known drugs can be reused with different tools or for a different purpose. This highlights the possibility, by exploiting nanomedicine, to design tailor-made treatments for each patient, thus corroborating precision and personalized medicine at the same time.

The major challenge of next years will be to bypass the defects of these novel approaches and eventually to promote the translatability of therapies. In this context, even if there is the possibility to generate very innovative and complex supermolecular NPs, the simplicity and biomimicry in NP design seems to be more successful in the context of scale-up and clinics.

Executive summary

Immunotherapy for glioblastoma multiforme

- Immunotherapy has become a standard treatment for tumors, glioblastoma multiforme included, because it prevents metastasis and recurrence. This therapy exploits vaccines, antibodies, oncolytic therapies and immune checkpoint inhibitors.
- The brain is a peculiar organ, protected by the blood–brain barrier and with a noncanonical lymphatic system. Antigens and other molecules flow throughout the brain in a network of meningeal lymphatic vasculature (glymphatic system) that is critical to initiate an immune response.

Nanoparticles as drug-delivery system

- Nanotechnologies, in particular nanoparticles (NPs), are valuable tools for the treatment of glioblastoma multiforme, a currently incurable CNS malignancy.
- Nonspherical and biomimetic NPs seem to have a long half-life, improved cellular uptake and biodistribution, a greater ability to cross biological barriers, blood–brain barrier included, in comparison with their spherical counterparts.
- Polymeric NPs, such as PBAE-based NPs and solid lipid NPs, are particularly useful for delivering nucleic acids (RNAs, siRNAs and miRNAs). Other nonviral vectors include cationic liposomes which are able to efficiently infiltrate tumors. Viral vectors can be used as well, thanks to their ability to infect cells.

Alternative strategies

- Chimeric antigen receptor T cell cells are engineered T lymphocytes expressing artificial antigens and they are a well-established strategy for tumor immunotherapy, although many patients do not respond to the treatment.
- Combining immunotherapy and radiotherapy is a valid approach for patients who do not respond to antiprotein death 1/protein death ligand 1 therapies, since radiotherapy increases the expression of these receptors in cells.
- Doxorubicin-polyglycerol-nanodiamond composites have shown to be an alternative strategy to deliver already known drugs to tumors using novel technologies.

Author contributions

Each author has contributed to the written text. L Taiarol read and evaluated the references and wrote the manuscript. F Re conceived the original idea, supervised the work and reviewed each version of the manuscript. B Formicola and RD Magro contributed to the revision of the manuscript. S Sesana reviewed the final version and handled the graphic layout. All authors provided critical feedback.

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